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Review

A Good Life for Laboratory Rodents?

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Abstract

Most would agree that animals in research should be spared "unnecessary" harm, pain, or distress, and there is also growing interest in providing animals with some form of environmental enrichment. But is this the standard of care that we should aspire to? We argue that we need to work towards a higher standard—specifically, that providing research animals with a "good life" should be a prerequisite for their use. The aims of this paper are to illustrate our vision of a "good life" for laboratory rats and mice and to provide a roadmap for achieving this vision. We recognize that several research procedures are clearly incompatible with a good life but describe here what we consider to be the minimum day-to-day living conditions to be met when using rodents in research. A good life requires that animals can express a rich behavioral repertoire, use their abilities, and fulfill their potential through active engagement with their environment. In the first section, we describe how animals could be housed for these requirements to be fulfilled, from simple modifications to standard housing through to better cage designs and free-ranging options. In the second section, we review the types of interactions with laboratory rodents that are compatible with a good life. In the third section, we address the potential for the animals to have a life outside of research, including the use of pets in clinical trials (the animal-as-patient model) and the adoption of research animals to new homes when they are no longer needed in research. We conclude with a few suggestions for achieving our vision.

Key words: agency; animal welfare; complexity; free-range; natural behaviour; socialization

Introduction

The relationship between humans and animals in laboratories is ambiguous. On the one hand, animals are brought into research institutions as a means to an end; they are the tools of scientific enquiry and their use is justified on the basis of benefits to us [1]. But they are also sentient individuals with whom we sometimes share our homes and form strong and long-lasting relationships.

The near-universal consensus is that animals in research should be spared "unnecessary" harm, pain, or distress [2, 3]. In practice, this usually means ensuring the animals are adequately fed and watered, kept relatively free of disease, and given pain relief when needed. Species-specific requirements, such as social housing and some form of environmental enrichment, are also considered; however, these are weighed against other concerns and provided if they are perceived to be practical and not interfere with the study aims [4–6]. But is this the standard of care that we should aspire to? We argue that we need to work towards a higher standard—specifically, that providing research animals with a "good life" should be a prerequisite for their use. There are several potential arguments for this position, but here we touch on just 4. First and foremost, we suggest that it is our duty, under the terms of the "ancient contract," to provide a good life to animals we have taken under our care and from whom we expect to benefit [7]. The other 3 arguments are pragmatic: keeping animals in better conditions is likely to make the results of the research more generalizable and more repeatable [8, 9]; caring for animals who are living their best life may increase job satisfaction and decrease compassion fatigue in animal care staff [10]; and, given that societal expectations regarding the standard of care for animals are increasing, having a high standard of care may better allow animal researchers to continue to justify their use (ie, retain their social license to use animals for scientific progress).

The aims of this paper are to illustrate our vision of a "good life" for laboratory rats and mice and to provide a roadmap for achieving this vision. We focus on rats and mice because these rodents comprise a large proportion of animals used in research, and the standard of care that they receive is sometimes lower than that provided to other mammals [11].

This paper focuses on the quality of the animals' day-to-day living conditions and how this contributes to a good life. We recognize that several research procedures are clearly incompatible with a good life, such as inducing disease or exposing conscious animals to repeated or prolonged aversive procedures. Harms experienced by animals as a result of the study can constrain or even preclude the ability for these animals to have a good life. We describe here what we consider to be the minimum daily living conditions to be met when using rodents in research; these conditions will help to provide animals with as good a life as possible within the research setting. Any specific suggestion may not apply to all research models; the aim is to describe general principles that can be tailored to fit a range of situations.

What is a "Good Life"?

According to one popular model, good animal welfare lies at the intersection of biological functioning, affective states, and natural living [12]. These 3 domains are interconnected such that changes in 1 sphere tend to affect the other 2. For example, a mouse in a negative affective state (affective state) may have a compromised immune system and higher risk of cancer (biological functioning) and perform abnormal behaviors (natural living). The negative affective state may itself have arisen from housing conditions that thwart the ability to perform highly motivated behaviors (natural living) or from a genetic predisposition (biological functioning). Although a balance of the 3 domains is key to good welfare, different stakeholders tend to prioritize different domains [13]. Within the biomedical research community, biological functioning is usually the most important consideration. Efforts are made to ensure that animals have minimal exposure to pathogens and grow well (although less emphasis is sometimes placed on other aspects of biological functioning, such as normal metabolism [14]). In contrast, animal welfare scientists tend to place emphasis on the animals' subjective experiences [15], considering if positive affective states outweigh the negative ones. For the public, natural living is often most important, irrespective of species or context of animal use: they want to see cows on pasture and chimpanzees in the jungle [15, 16].

The concept of a "good life" extends beyond these 3 traditional domains of animal welfare. Philosopher Martha Nussbaum [17] argues that each individual—human or nonhuman—has dignity and must have the opportunity to flourish "as the kind of thing that it is". Individuals must be allowed to utilize their innate abilities and experience "a rich plurality of life activities". The focus is not only on health or happiness but also on whether they are living to their potential.

Related to Nussbaum's capabilities approach is the recognition that engaging with the environment, learning, and overcoming challenges are central to a good life. Wemelsfelder [18] argues that quality of life depends on opportunities that animals have to pursue their own interests and encompasses the animals' relationship with their environment and how they live their life. Purves and Delon [19] suggest that a meaningful life stems from intentional actions, or the animals' own agency. According to Špinka and Wemelsfelder [20], agency is important to animal welfare both as a process and as an end. Špinka [21] further proposes 4 tiers on the "agency" scale: *passive/reactive agency*, where animals exert behavior in direct reaction to external stimuli; *action-driven agency*, where animals engage in active behavior to achieve outcomes; *competence-building agency* that comprises active skill building and information acquisition for later use; and *aspirational agency*, where animals actively pursue planned and reflected goals. Špinka argues that engaging in action-driven agency and competence-building agency is associated with positive affective states and may contribute to the expression of the animals' full, species-specific potential.

We conclude that a pluralistic conception is required, one that attends to each of the features described above. Specifically, we argue that a good life requires that animals are able to express a rich behavioral repertoire, use their abilities, and fulfill their potential through active engagement with their environment. One benefit of this pluralistic conception is that it attends to features ignored by more unitary conceptions and thus is more likely to meet various lines of evidence for what constitutes a good life and to satisfy the range of public perspectives [22].

We have considered what a good life for laboratory rodents might look like from 3 perspectives: physical environment (how they are housed); interactions with humans; and life beyond research (life outside of their role as research subjects). Within each of the 3 perspectives, we have identified elements that contribute towards one or more aspects of the good life conception. Figure 1 provides a roadmap for implementing these various elements, and these elements are described in more detail in the sections that follow.

The Physical Environment

Standard Cages

The size and shape of standard laboratory cages for rodents are based more on tradition than scientific evidence [23–25]. The "shoebox" cage was primarily designed for convenience of handling and cleaning [24, 26, 27], and—at least in North America—has changed little since the 1920s [28, 29]. Providing rodents with a good life within the confines of a standard laboratory cage may not be feasible, but some features can be tailored to improve welfare.

Bedding. The chief function of bedding is to absorb moisture from the animals' excrement to minimize the build-up of ammonia and bacteria [30]. Bedding must also be nontoxic and comfortable for the animals to rest on [31, 32]. Corncob bedding is the most absorbent [30], and is associated with the lowest levels of ammonia [32]. However, cages where mice lived on corncob bedding contained acetic acid and sulfur dioxide, which are known eye, skin, and respiratory tract irritants [32]. Moreover, rodents seem to dislike corncob bedding. In a series of tests, rats and mice preferred to rest on aspen chip over corncob bedding [31, 33]. Leys and colleagues [34] found that rats showed less slow-wave sleep on corncob compared with aspen chip bedding. The authors also observed that rats would sleep directly on the aspen chip bedding but pushed corncob to the side and slept on the cage floor. In mice, corncob bedding has also been associated with higher levels of aggression [35], perhaps because it contains estrogen disruptors [36].

Blom and colleagues [37] assessed rat and mouse preferences for different types of bedding, excluding corncob. They found that animals preferred to rest in cages with shredded filter

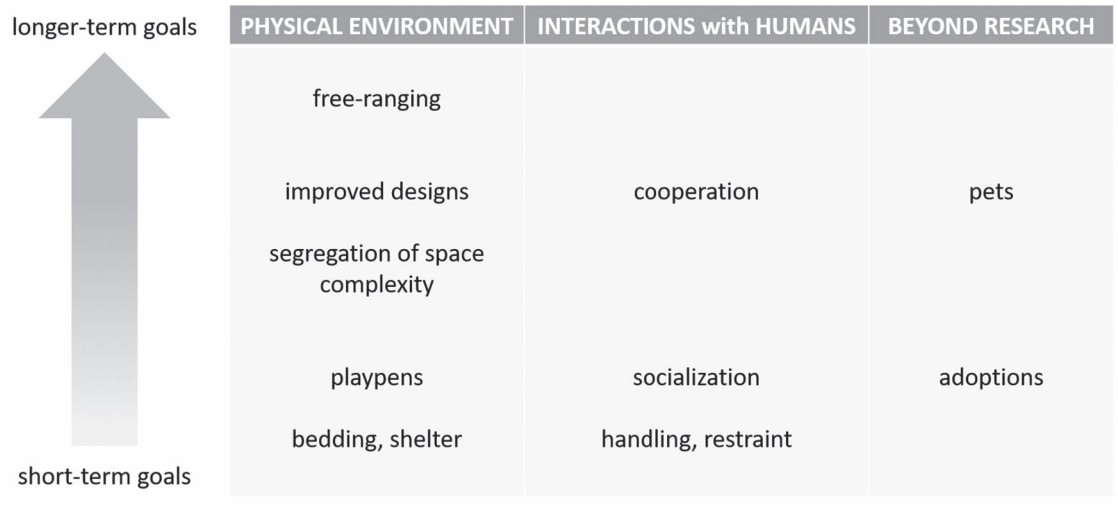

Figure 1: Roadmap to a good life for laboratory rodents.

paper, chose wood chips if shredded paper was not available, and avoided cages with sawdust. In a separate study, rats living on aspen chip bedding exhibited more sneezing and lung pathology compared with those living on virgin loose pulp bedding (a paper-based bedding) [38].

These data suggest that paper-based bedding may be the most comfortable for the animals to lie on and may also be associated with fewest deleterious health effects despite having low (eg, reclaimed wood pulp, such as carefresh and TEK-Fresh) to moderate (eg, virgin loose pulp, such as Alpha-Dri and Omega-Dri) absorbency [30, 32]. One solution to ensure low ammonia levels within cages bedded with paper-based bedding is to increase cage-changing frequency, but this may be disruptive to the animals [39], particularly when performed during the light phase of the light cycle [40, 41]. A better solution is to provide deeper bedding, which is associated with lower intracage ammonia [42, 43]. For example, Freymann and colleagues [43] tested the impact of 0.5-cm, 1.5-cm, or 6-cm depths of aspen chip bedding on male and female C57BL/6 and BALB/c mice. In all cages, ammonia levels decreased as bedding depth increased; 7 days after cage changing, no ammonia was detected in the cages with deepest bedding. All mice kept on the deepest bedding had higher body temperatures and lower food intake, while females of both strains had increased food conversion efficiency and lower corticosterone levels. Mouse preference for bedding depth was also assessed: all mice had a strong preference for the deeper bedding.

Shelter. The provision of adequate shelter and nesting material to laboratory rodents contributes to a good life on several levels. Manipulating nesting material and seeking cover offer burrowing rodents the opportunity to express these natural behaviors. Moreover, building and retreating into a shelter provide opportunities for rodents to actively engage with their environment. Building a nest allows the animals to create something they will later use—this constitutes an example of Špinka's [21] action-driven agency and may also fulfill animals' prevention motivation (ie, a type of motivation to attain non-losses, such as securing and maintaining safety) [44]. Retreating into a nest or shelter allows rodents to control their exposure to perceived threats, bright lights, and ambient temperatures [45–48]. Finally, nest boxes offer an additional behavioral opportunity: they can be used as an elevated resting surface. Indeed, rats are often observed sleeping inside the nest box during the light period and resting on the nest box during the dark period [49–51].

Some researchers have suggested that nest building is an acquired behavior in rats [52], but others observed that naïve rats will build nests as long as suitable resources are available [53], or when they are highly motivated, such as when pregnant, nursing, or under thermal stress [23, 48]. In the wild, Norway rats dig tunnels that lead to nesting chambers, which they line with a variety of organic materials [53–56]. Eibl-Eibesfeldt [53] described that the first step in rats' nesting process is the building of a nest chamber or the presence of a secure sleeping area. There is some evidence that rats prefer a good nest box vs good nesting material but that having both is preferred [57]. Providing laboratory rats with a suitable nest box as well as appropriate nesting material would thus allow better expression of nesting behavior. Indeed, laboratory rats offered both resources often carry the nesting material inside the nest box [49, 58].

Both the nest box and nesting materials must be appropriate for rats to use them. One common type of nest box for rats is the open-ended tunnel, but rats appear to have little use for these [50, 59–61] and prefer instead enclosed nest boxes with only 1 open end [49, 50]. Rats prefer shelters that are dark, opaque, and made of Plexiglas over shelters that are clear or made of cardboard or tin [62], and prefer nesting materials that consist of long (40 cm), wide (1 cm) paper strips over short (<2 cm) paper or wood shavings [49].

Like rats, wild mice generally build nests inside a protected area, such as an underground burrow, but will build a surface nest if suitable protected area is unavailable [46, 63]. Laboratory mice bring nesting material into a suitable nest box if possible, but choose nesting material over a nest box if forced to make a choice [64–66].

Mice prefer facial tissue paper or paper towel over paper strips (Enviro-Dri) and generally prefer paper-based over wood-based materials for nesting [67]. Nest quality is better when mice use paper strips compared with facial tissue paper or compressed cotton squares (Nestlets) [68]. When more than 1 type of nesting material is provided, mice will build layered nests using different types of material [67, 68]. For example, Hess and colleagues [68] found that mice provided with facial tissue and paper strips lined their nests with shredded facial tissue and used the paper strips to create an outer, structural layer.

Mice prefer nest boxes that are rectangular rather than circular [66], and with 1 vs 2 openings [69]. They also choose boxes made of perforated material (eg, perforated metal or grid metal), presumably because this design allows the passage of olfactory cues [66, 69]. In one study, mice showed a strong preference for Shepherd Shacks over the Tecniplast Mouse House, but more research is required to determine which features of the latter are preferred, as the 2 shelters are made of different materials (opaque cardboard vs red transparent Perspex) and are shaped differently [65]. There is considerable variation in nest box choice between individuals [66], and many choose to divide their time between 2 boxes [69].

Complexity. Environmental complexity provides animals with opportunities to interact with their surroundings, express a range of behaviors (eg, exploration, climbing), and exert another level of control over their lives (eg, choosing what to do and in which area of the cage) [70]. In general, rodents have a preference for and show more indicators of good welfare in more complex environments [50, 71, 72]. Although standard shoebox cages are small, it is possible to add some complexity as explained below.

Van der Harst and colleagues [73, 74] developed a set of tunnels and platforms that increased the usable area inside a standard rat cage by 45% (Figure 2). Their cage contained a shelter with a flat top, as well as another shelter-like structure with large circular openings on the top and on the sides, and small openings through which gnawing sticks were inserted. The cage also contained a short pan along the width of the cage that rats used as a toilet, and a raised lid that added 8 cm of vertical space (the raised lid was custom-made, but raised lids are now commercially available). These structures increased usable space, provided opportunities to withdraw from conspecifics or perceived threats, and allowed the animals to use the elevated platforms as a lookout. Van der Harst and colleagues showed that standard-housed rats found access to this cage as rewarding as sexual contact and more rewarding than access to another standard cage [74]. Stress and deprivation of essential stimuli lead to increased sensitivity to rewards, and this increased sensitivity to rewards can be measured by differences in the level of anticipation for a reward [75–78]. Rats living in these cages showed less anticipatory behavior for a sucrose reward than did standard-housed rats [73], indicating that standard-housed rats were "impoverished" compared with rats housed in these more complex cages [78, 79].

A simple and relatively inexpensive way of increasing the complexity of a standard laboratory cage is through the addition of cage dividers. Anzaldo and colleagues [80] found that male rats preferred a cage with 2 L-shaped dividers compared with no dividers. In a more elaborate experiment, Chamove [81] partitioned standard mouse cages into 5 or 9 alleys using a combination of transparent and opaque Perspex. The dividers, inserted lengthwise, had small cut-outs at alternating ends, such that mice had to travel the length of the cage 5 or 9 times to get from one end of the cage to the other. A third set-up, dubbed the savannah cage, had 9 alleys but was also divided horizontally halfway through its height with a flat piece of Perspex. As a result, there were "burrows" in the lower half and an open "savannah" in the upper half of the cage. The horizontal divider had a small hole so that mice could travel between the 2 levels. In preference tests, male and female mice had a clear preference for increasing complexity, with the savannah cage being the favorite. Mice reared from weaning in the more complex cages had better weight gain after weaning, lower adrenal weights, were more active in their home cages, had shorter latencies

Figure 2: van der Harst and colleagues added structural complexity and 45% more usable space by furnishing rat cages with tunnels, shelters, and a raised lid. Photo printed with permission from Johanneke van der Harst.

to emerge from a novel box, and in the open field test walked more, defecated less, and groomed less. These measures were better in the 9-alley cage compared with a control cage or the 5-alley cage, but there were few differences between the 9-alley and the savannah cages. Animal care staff noted fewer fights and vocalizations in the 9-alley cage. Haemisch and colleagues [82], who also designed a sort of savannah cage, noted more aggression in this cage towards intruders, but not towards cage mates, compared with a standard cage.

Tallent and colleagues [83] developed a simple cage divider: a corrugated plastic or fiberboard insert that divides one half of a mouse cage into 3 narrow sections. They found that male BALB/c mice housed in these divided cages had fewer aggressive events, including posturing, scuffling, and unprovoked biting. They also noted that mice built their nest in 1 of the 3 narrow sections, where they slept together. When a mouse would begin chasing another mouse aggressively, escape to or from one of the narrow sections would end the aggressive event. In a follow-up study, they found that male C57BL/6 mice housed in these partitioned cages had better weight gains, fewer bite wounds, and showed less evidence of anxiety [84, 85]. When introducing cage dividers into a mouse cage, it may be important to consider a design that offers several escape routes: this most closely resembles mouse burrows, which always contain several openings to the surface, and this design prevents 1 mouse from cornering another and blocking the only escape route [82].

Godbey and Gray [86] created a 2-tiered cage for mice by stacking a mouse cage into a rat cage of similar length and width (Figure 3). They drilled a hole into the floor of the top cage at one or both ends and filled the 10-cm space between the 2 cages with bedding substrate (wood shavings, paper bedding, or straw). Godbey and Gray observed that mice slept in the bottom cage during the light phase and used the top cage during the dark phase. Pregnant females would give birth and keep their young in the bottom cage [87]. If a tunnel or other structure was buried in the bedding, mice would burrow down to it, creating a tunnel system (Tamara Godbey, personal communication).

Segregation of Space. Much attention has been paid to minimizing ammonia levels, but little work has assessed if laboratory rodents are motivated to avoid contact with their urine and feces even when ammonia levels are kept to an acceptable level. New types of bedding and ventilated caging have allowed cages to be changed less frequently, increasing the relevance of this issue.

Makowska and colleagues [88] showed that mice are motivated to nest away from the area where they eliminate: when housed in a system that facilitates spatial segregation, female

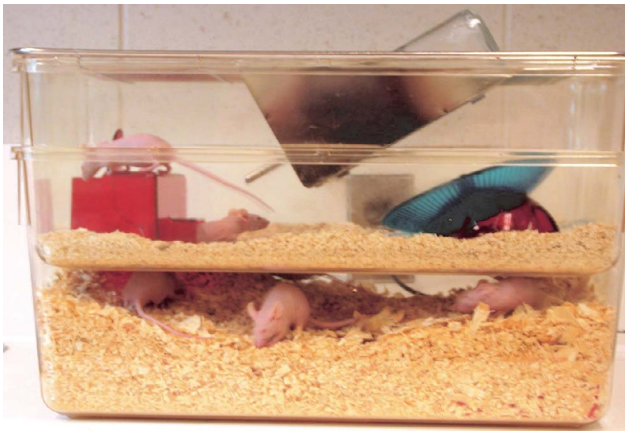

Figure 3: Godbey and Gray created a 2-tiered mouse cage by stacking a mouse cage into a rat cage and filling the bottom cage with bedding substrate. Photo printed with permission from Tamara Godbey.

mice placed a larger distance between their nesting and soiling sites and carried the bulk of their bedding material from the nesting and neutral sites into the latrine area. Mice also avoided urinating and defecating close to their nest. The scientific literature abounds with anecdotal evidence that mice prefer to segregate their space into clean and dirty areas. For example, Sherwin [89] observed that mice will defecate in a highly localized area when provided with a demarcated space, such as a glass dish. Others have observed that when empty bottles were added to a mouse cage as novel environmental enrichment, mice would use these as enclosed latrines or nesting areas [90, 91]. In studies on mouse preferences for bedding, mice were found to eliminate least in the cages that contained their preferred bedding type (ie, where they rested) [37]. Anecdotal evidence suggests that rats also prefer to eliminate away from areas where they rest [37, 55, 72, 74].

Providing rodents with opportunities to segregate clean and dirty areas is likely an important welfare consideration. Establishing separate resting and soiling sites allows animals to engage in this naturally motivated behavior and provides them with the opportunity to exert some control over their environment. As reviewed above, a simple method of promoting the segregation of space into clean and dirty sites is to add a demarcated area in the animals' home cage. Mice [92] and rats [93] prefer to eliminate near their food and water; this should be taken into consideration when designing cages. For example, a litter pan could be added to cages near or under the food hopper. The litter pan could be filled with absorbent bedding, allowing the use of more comfortable bedding elsewhere in the cage.

An effective means of providing rodents the opportunity to segregate space is to connect 2 or more cages via tunnels [88]. Such systems are available commercially or can be made in-house by connecting existing cages with short pipes [94, 95]. As noted earlier, one may consider connecting 2 cages with more than 1 tunnel for aggressive strains of mice to provide several escape routes and minimize the opportunity for a dominant individual to monopolize the tunnel. For rats and female mice, we suggest providing food and water in only 1 cage—the animals will likely use this as a latrine (this cage could be bedded with absorbent bedding) and establish their nesting/resting sites in the other cages (that could be bedded with more comfortable bedding). This system allows cleaning only the latrine cage, resulting in less disturbance to the animals [88].

Figure 4: Semi-naturalistic cage for rats with multiple levels and burrowing soil. Photo by Joanna Makowska.

Improved Designs

According to our conception of a good life, a well-designed rodent cage should allow the animals to express a wide range of natural behaviors and provide multiple opportunities to engage with, and have some control over, their environment. In nature, both mice and rats use burrows [55, 63]. Despite many generations of captive breeding, laboratory rats [96] and mice [97] still build burrows when provided a suitable substrate, and there is evidence that excavating burrows is a highly motivated behavior in both species [98, 99].

Makowska and Weary [98] designed a semi-naturalistic cage for laboratory rats that included soil for burrowing (Figure 4). The multi-level cage for pet rats (Critter Nation double unit with stand, MidWest Homes for Pets, Muncie, IN) was furnished with tunnels, climbing structures, a hammock, and soil for burrowing in the lower portion of the cage. Rats housed in groups of 5 performed a range of behaviors that are not possible in a standard laboratory cage, such as burrowing, climbing, and standing and stretching upright [98]. In these cages, rats spent the bulk of the light period in their burrows; during the dark period, they rested in the hammock between bouts of excavating their burrows (each rat did this approximately 30 times per day) and various forms of exploration. Using anticipatory behavior as a measure of underlying affective state, Makowska and Weary [100] found that these rats fared better than pair-housed control animals housed in standard laboratory cages.

Soil (eg, black earth, peat moss) is a natural burrowing substrate that allows rodents to build tunnels that do not collapse [96]. Soil can be autoclaved (sterilized) when it is brought into a biosecure research facility (personal experience), but it can be messy, so researchers may wish to consider alternatives. One option is to keep the soil inside a large container closed by a lid

with a small circular opening (Megan LaFollette, personal communication). Another option is to substitute soil for wood wool (also known as excelsior), which can also be autoclaved. Wood wool does not allow rats to excavate tunnels through digging, but tunnels can still be formed when rats muzzle through it (personal observation).

Large cages furnished with multiple, biologically relevant items have been used by other researchers. Clarke and Ioannou [101] developed gang caging for rats using existing caging for ferrets (Figure 5). Two multi-level cages were connected using a polyurethane tube. Each cage was furnished with suspended and floor-based PVC tunnels, aspen wood chew blocks, and a large nest box. Rats housed in this system were calmer, easier to handle, and did not startle when someone entered the housing room. The rats displayed species-specific hopping gait, climbing, jumping, nesting and cooperative hoarding, and extensive foraging.

Working with mice, Slater and Cao [102] designed a housing system in large bins (120 × 90 cm). Each bin housed 10 to 20 mice and contained a multitude of objects, including wooden logs, tunnels, igloos with saucer wheels, and running wheels. Food and water were provided from the wire lids of 2 standard mouse cages placed inside the large bin (a circular hole was drilled into one side of each standard cage to allow entry). The items inside the bin were rearranged weekly. Mice living in these large and structurally complex environments had less fat, stronger immune systems, and lower serum corticosterone compared with standard-housed animals. Moreover, when the mice were injected with cancerous cells, tumors took longer to develop, and these tumors were smaller than those in standard-housed mice [103]. The authors speculated that the dynamic social interactions, frequent exposure to novel objects, and increased physical activity led to mild and beneficial activation of the HPA axis ("eustress"), helping to buffer negative stressors [103–105].

Marlau cages are commercially available and combine social housing in large groups with opportunities for exercise and cognitive stimulation. The cages are relatively large (rats: 80 × 60 cm and 51 cm high; mice: 58 × 40 cm and 32 cm high) and accommodate up to 12 rats or 18 mice. The space is divided into 2 floors; the ground floor is further divided into 1 larger section with running wheels and water bottles, and another smaller section with food. To access the smaller food section, animals must climb a ladder to the upper level, travel through a maze, and slide down a tunnel into the smaller section of the ground floor. From this food section, animals can walk through a 1-way door into the larger section with water and running wheels. There are 12 configurations to the maze [106]. Rats raised in Marlau cages from weaning had better learning and memory, decreased anxiety in a novel water exploration test, a black/white/black box, and the elevated plus maze and faster recovery of basal plasma corticosterone after acute restraint [107].

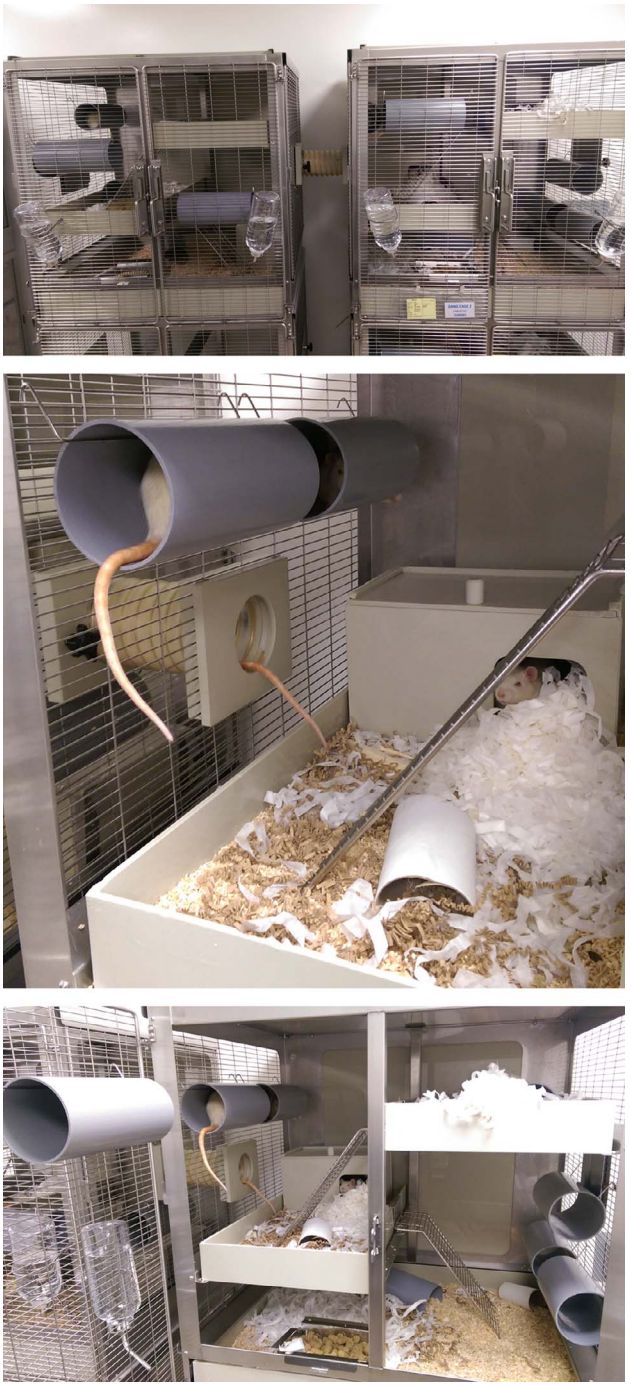

Figure 5: Gang caging for rats. Reproduced with permission from: Clarke, D., Ioannou, L. 2018. Introduction of gang caging for group housed rats. *Animal Technology and Welfare* 17(2):136–137.

Playpens

Animals may also benefit from regular access to a "playpen." Playpens are large and structurally complex enclosures to which animals are provided intermittent access. The idea is to provide animals with opportunities to socialize, exercise, and express a wider range of natural behaviors through interactions with a structurally rich environment.

The concept of playpens is not novel: nonhuman primates, dogs, and rabbits have long been provided with such opportunities. In a book chapter on environmental enrichment for nonhuman primates, Reinhardt [108] describes several studies (some published in the 1980s) assessing the benefits of providing standard-housed nonhuman primates with daily access to a large, environmentally complex pen. While in these large, enriched enclosures, macaques and baboons exhibited fewer stereotypies and self-directed aggression, including self-biting and hair pulling. Similar interventions and benefits have been described for rabbits [6, 109, 110] and dogs [87, 111, 112].

Deacon [113] described his routine use of playpens for rats, claiming that this resulted in animals who were easier to work

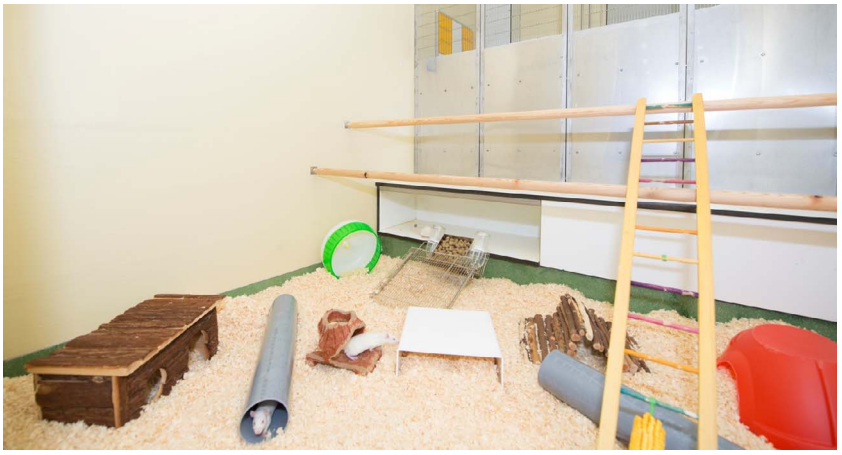

Figure 6: Playroom for rats. Groups of up to 10 rats were rotated through the room every week. Photo printed with permission from Timothy Jones.

with (especially for inexperienced students) and seemed more emotionally stable and better able to adapt to new experimental tasks. Young (100–200 g) rats were pair-housed in standard cages but placed in a large playpen for 2 to 6 hours each day in groups of approximately 30 animals. The rats were then returned to their home cages in random pairings. No aggression was observed, likely because the rats were busy engaging with the stimulating environment. The playpen floor measured 80 × 45 cm and was 35 high; it contained tree branches, ramps and ladders, pipes, chains, metal coils, and hanging baskets. After playing, the rats retired to the pipes, allowing the animals to be moved with relative ease.

Similarly, Shenton [114] described her use of a playpen for rats, consisting of a spare rabbit cage furnished with water trays, ladders, ropes, and tunnels. Treats were scattered throughout the pen. Pair-housed male rats were placed in groups of 8 to 12, 2 to 3 times per week for an average of 30 minutes. Similarly to Deacon, no aggression was seen. Animal technicians noted that the rats were calmer and more confident and interactive when handled.

Tim Jones (personal communication) set up an entire playroom where groups of up to 10 standard-housed rats were kept for 1 week at a time (Figure 6). The floor in the playroom was covered with a deep layer of wood shavings, and a litter box with corncob bedding was provided in 1 corner. The litter box was cleaned daily, but the rest of the room needed only spot cleaning. The playroom was furnished with various hiding items as well as 2 horizontal poles suspended across the room and accessible via a ladder. There was also a large digging box filled with soil that rats used extensively.

In our laboratory, we have provided standard-housed rats with access to a playpen that includes a burrowing substrate. Initial data suggest that rats find access to the playpen rewarding. We are currently assessing the use of playpens for mice and suggest that the benefits of regular social interaction, exercise, and interaction with a more stimulating environment also extend to this species.

Free-Ranging Animals

Keeping animals in a more naturalistic environment may help provide several elements of a good life: a naturalistic environments affords an opportunity for animals to express a rich behavioral repertoire, utilize their abilities, and have the chance to fulfill their potential through active engagement with the environment. A naturalistic environment may allow animals to establish their own territory, form alliances with chosen conspecifics, forage for preferred food types, build nests with the materials of their choice, and occupy their day with activities they deem most important. In an article entitled "Unbridle biomedical research from the laboratory cage" [115], behavioral neuroscientist Lahvis argued that captive animals are abnormal and make poor models for studying human health. Lahvis also suggested that, to increase relevance for human health, research animals should either live in the wild or roam free in naturalistic captive environments where they can experience a normal range of experiences—both positive and negative.

The use of free-ranging animals may be relatively straightforward for certain types of research, such as that on lemurs at the Ranomafana National Park in Madagascar. Lemurs develop many of the same diseases as humans, such as diabetes and Alzheimer's. To study the progression of these conditions in wild animals, Wright and colleagues have implanted computer chips in hundreds of lemurs. They have suggested that this work will provide improved translation in the development of new drugs for humans [116].

Studies of free-ranging animals are also taking place at Cayo Santiago, an island off Puerto Rico, home for approximately 1600 rhesus macaques [117, 118]. The monkeys are descendants of 409 individuals first brought to the island in 1938. The colony is maintained by the University of Puerto Rico for behavioral and noninvasive biomedical research. The animals are provided with a commercial diet, but 50% of their eating time is spent foraging on the island's vegetation [119]. The monkeys self-organize into social groups that fluctuate in number and size. Once a year, yearlings are caught, genotyped, and marked for visual identification [120].

The study of these free-roaming macaques has yielded insights into the management of infectious diseases and the effectiveness of immunization programs [121], the genetic basis for sociality and related disorders [117], such as autism spectrum disorder [122], and a variety of age-related diseases [120]. The animals are also used for cognitive research. Researchers concede that studying free-roaming animals has its challenges but feel that these are outweighed by the benefits. In laboratory-based behavioral research, individual animals can be physically isolated, but on Cayo Santiago the animals' participation is voluntary, requiring researchers to modify their research methods. In the laboratory, researchers provide monkeys with food rewards to keep them motivated in the task; for this to be

Figure 7: Free-ranging housing options for mice. Left: König conducts experiments with mice living in a barn; mice are free to enter or leave the barn at will through small holes in the walls. Right: Graham houses mice in fenced outdoor enclosures where they can build burrows and forage on vegetation. Mice cannot leave the enclosures.

successful, the animals are food restricted and fed a significant portion of their daily ration during testing. Free-roaming monkeys are able to eat whenever they want, so researchers use methods that do not require the use of food rewards, such as the looking time method (individuals will gaze longer at events that differ from physical or social norms) that is minimally disruptive and was developed for use with human infants. Using the same method with monkeys as is used with infants also provides a better basis to compare performance between species [123].

Free-ranging rodents have been used in similar ways. In 2002, König and Weidt captured 12 wild house mice from 2 populations and placed them in a barn (floor space 72 m^2) in northern Switzerland. In this barn, the concrete floor is covered with commercially available rodent bedding and the mice have access to 40 fabricated nest boxes, straw, and nesting material as well as bricks and various barriers to provide structure (Figure 7). Water and food (50% commercial rodent food and 50% oats) are provided in various feeding trays throughout the barn. The barn is open to emigration and immigration—mice are free to leave or enter the barn under the roof or through small holes in the walls. As such, the barn is free of predators but not parasites [124]. Mice living in the barn—approximately 300–400 individuals at any one time—are implanted with miniaturized passive-integrated transponder tags, allowing the researchers to monitor animals remotely, including use of nest boxes or drinking trays [125].

König's research is mostly observational, focusing on social, reproductive, and maternal behavior. However, her group has also used this mouse population for experimental work. First, the animals' social networks were determined based on data documenting which individual mice shared nest boxes. Then, mice from different social groups were injected with saline as a control, or lipopolysaccharides (a proinflammatory agent) to induce short-acting (48 hours) lethargy, a common symptom of infection. Postinjection changes in social networks were then examined. The goal of the study was to investigate the effects of inflammatory challenge on social dynamics to better understand this component of sickness behavior [126] and the effects of sickness behavior on the potential for disease spread [127]. To catch and inject the mice, the researchers placed glass jars at the entrance to target nest boxes: the animals would hide in the nest boxes when the researchers entered the barn, at which point the jars were put in place and the mice were caught as they exited the nest box [126]. The daily capture of all focal mice (3–4 individuals per day) took on average 24 minutes [127].

The ability for research animals to leave the study site and interact with wild populations, as described above, may not be desirable for studies that require higher degrees of experimental control. For example, part of König's work was run concurrently with a laboratory component (housing in this case consisted of large indoor enclosures) to give researchers the ability to manipulate relatedness of the animals and film them outside of the nest boxes. Additionally, comingling with wild animals could impose risks on both the wild and research populations. Animals who are genetically modified must not be allowed to breed with wild-type populations, and animals who are physically, physiologically, or immunologically compromised could be at high risk of mortality if exposed to predators or parasites.

Graham, an ecological and evolutionary immunologist, has conducted experiments with mice living in circular outdoor enclosures since 2015 [128]. The fenced enclosures, which are divided into wedge-shaped pens each measuring 180 m^2 and housing up to 25 mice, are designed to keep mice in and predators out (Figure 7) [129]. Mice excavate burrows in the soil and feed on insects and vegetation, such as berries and seeds. To supplement the animals' housing and dietary needs, pens also contain a straw-filled shed, 2 watering stations, and a feeding station with standard laboratory chow.

Potts, a molecular biologist, has been conducting experiments with mice living within a fully enclosed barn since the 1980s [128]. Groups of about 30 mice live inside 30–35 m^2 enclosures that are divided into high-quality (containing dark, enclosed nest boxes) and low-quality (containing bright, open-top nest boxes) territories [128, 130]. Male mice compete for and defend territories, and those who are more successful attract more mates [128]. Mice are implanted with passive-integrated transponder tags that allow researchers to monitor their visits to feeding stations. Potts claims that this housing allows him to perform more sensitive drug toxicology tests. For example, female mice given the drug cerivastatin (a statin) in their daily food had 25% fewer offspring, and males had 41% fewer offspring, occupied 63% fewer territories, and had 10% lower body mass [130]. This drug had been found to be safe using traditional laboratory testing in mice (and other species) [131] but was subsequently pulled from the market due to serious health complications in humans [132].

One limitation of Potts' system is that it does not allow keeping mice on different diets [133]. Therefore, Potts first houses mice within traditional laboratory settings where they are given different diets, and these treatments end once the animals are

released into the barn where they all eat the same food, compete for territories, and reproduce. Newer technologies can allow researchers to control individualized feeding for free-ranging animals. For example, our research group uses this technology to control access to diets by loose-housed dairy cows. Feed bins are programmed to allow access to individual cows [134]. Similar technology could be used to allow mice from different treatments access to feeding stations containing specific (unhoardable) diets.

Other technologies can facilitate the study of free-ranging animals by aiding in data collection, animal tracking, and health assessments. For example, infrared thermography can be used to detect inflammation and to localize individuals and their habitats [135]. Infrared thermography has also been used to assess respiratory rate in rats [136]. Radio-frequency near-field coherent sensing was used to detect respiratory and heart rate in a hamster using a running wheel, a parakeet using a perch, as well as a tortoise and a fish from outside their glass tanks [137]. Another infrared-based device, a 3D time-of-flight camera, is capable of assessing the volume of subcutaneous tumors in moving mice [138]. Technology now also enables remote cortical imaging in awake animals; this is expected to extend to imaging changes in blood flow and other metabolic signals [139]. Thus, with some creativity and the use of appropriate technology, the study of free-ranging animals is possible.

Interactions with Humans

Rats and mice are inherently fearful of humans, yet we are a daily presence in their lives within the laboratory; even with free-ranging animals, some interactions are necessary. The type of interactions and the quality of our relationship with individual animals can have a profound effect on their life experience.

Handling and Restraint

Laboratory rodents interact with humans during routine procedures, such as cage changing and health assessments. These procedures usually involve handling and restraint. Using methods that give animals more control over these interactions would be more consistent with a good life. For example, Lipták and colleagues [140] modified the traditional procedure for restraining rats to record blood pressure with a tail-cuff: instead of confining rats to a small chamber, rats are placed on a table and gently restrained under a cover sheet. When tested with the cover sheet method, rats had lower mean arterial blood pressure and exhibited lower rates of struggling, defecating, and cheeping [140]. The time to obtain blood pressure readings was similar between the 2 restraint methods, averaging 217 s for classical restraint and 231 s for the cover sheet method [140].

Similarly, Stuart and Robinson [141] developed a modified technique for intraperitoneal injections. Compared with the conventional methods of scruffing (grasping the scruff of the neck between the thumb and index finger) or encircling (enclosing the upper body with one hand, and supporting the lower body with the other hand or by resting it on the handler's hip), the modified method was associated with lower plasma corticosterone, less struggling and defecation, fewer audible vocalizations, and a more positive affective state after the injection [141]. In the modified method, rats' lower body rested on the handler's hip while the upper body was only partly restrained from the back using the fingers of one hand. When picking up a rat, lifting by the base of the tail or encircling produce higher changes in heart rate and mean arterial blood pressure compared with scruffing [142].

Mice also experience stress and anxiety when they are picked up by the tail. Compared with lifting with a tunnel or cupped hands, male and female mice of various strains picked up by their tail show lower voluntary interaction with the handler [143–146], more urination and defecation during handling [143, 146], higher anxiety in the elevated plus-maze [143–145, 147] and the open field test [145, 146], higher stress-induced plasma corticosterone [147], and lower consumption of a sucrose reward, indicating a depressive-like state [145]. Mice picked up with a tunnel or cupped hands may subsequently be restrained by their tail—it is the capture by the tail that mice find aversive [143]. To use tunnel handling successfully, the tunnel should be grasped by its middle so that both ends remain open, and mice should not be chased with the tunnel—it should be placed along one of the cage walls and the mouse gently guided towards the tunnel with the free hand [148].

Socialization

Naïve laboratory rats experience stress when in close proximity to humans, showing an increase in serum corticosterone and heart rate when their cage is removed from the cage rack [149]. They also vocalize in the 22-kHz range (indicative of anxiety) when gently touched [150]. Over time, rats may *habituate* to routine handling and restraint, but this is not the same as being *socialized* and comfortable around humans. Indeed, rats whose only interaction with humans was during weekly cage changing and weighing were more likely to vocalize, freeze, or run away when approached or lifted by familiar or unfamiliar humans compared with rats who had been "gentled" [151]. Gentling consisted of softly touching newly weaned rats over their entire bodies, hand-feeding treats, lifting them twice, and talking to them in a friendly and soothing manner for 10 minutes twice a day for 2 weeks. Similarly, rats who had only been handled during routine husbandry procedures had higher basal norepinephrine levels and higher anxiety in the elevated plus-maze compared with rats who had been placed on a lap or table and gently stroked on the back and neck for 5 minutes, 5 times per week for 6 weeks [152].

Playful interactions with rats also further welfare. For example, engaging with rats in a playful manner that mimics their social play (often referred to as rat "tickling") decreases responses to various stressors, motivates rats to seek out human interaction, and is associated with the experience of a positive emotional state [153]. Playing hide-and-seek with rats is also seemingly enjoyed by both parties [154].

Although much fewer data exist on socialization for mice, there is some evidence that the quality and quantity of interactions with laboratory mice also affect their well-being. In 1 study, 3 groups of adult mice were subjected to 1 of 3 treatments every second day for 13 days: gentle handling (placed in the palm of a hand and stroked on the flanks and head for 90 seconds), aggressive handling (suspended in the air by the proximal end of the tail for 90 seconds), or control (handled during routine cage changes only). When tested in the forced swim test, gently handled mice spent the least time immobile, indicating lower behavioral despair compared with the other groups [155].

Cooperation

Training animals to voluntarily cooperate with husbandry, veterinary, and research procedures may be one of the most impor-

tant ways in which they can actively engage with and control their environment. There is some empirical evidence that engaging with the environment to control routine daily events can reduce anxiety and enhance exploratory behavior in laboratory animals [156, 157].

The training of animals to voluntarily participate in experimental procedures is increasingly common with nonhuman primates, where cooperation is seen to improve both animal welfare and scientific quality [158]. For example, macaques who were trained to voluntarily present a limb for blood collection or injection in the home cage had lower physiological [159] and histological [160] indicators of stress compared with animals removed from their home cage and restrained for the procedure.

Training laboratory rodents to cooperate with procedures is less common, but it is not without precedent. For example, rats have been trained to sit still on a weighing scale, enter a restraint tube, or participate in oral gavage of an unpalatable substance, and mice were trained to cooperate with limb shaving and saphenous vein draw [161, 162].

A cooperative approach affords several elements contributing to a good life. In addition to the opportunity for actively engaging with and controlling the environment, training also helps to build a relationship between the animal and handler, and this may reduce anxiety and aggression in other novel situations [160]. Finally, when a normally cooperative individual is suddenly reluctant to participate in a trained task, this may also help in early detection of other problems [160].

Life Beyond Research

The life of a typical research rodent is short: the animals are enrolled in a study soon after weaning and are killed a few months later, far short of their normal lifespan. If one aspect of a good life is fulfilling one's potential, then arguably this requires the opportunity to live a more normal lifespan. One way to allow these animals a longer (and likely better) life is to find them homes after their career in research is complete.

Adoptions

Many research institutions attempt to adopt out dogs (and sometimes cats) who are no longer used in research, but this practice is less common for laboratory rats and mice. These rodents can also benefit from a life outside of research. Many small rodents are kept as pets throughout the United States [163], and research institutions can be one source for people to procure these pets; for example, nearly 40–50 small animals are adopted into homes each month in Poland, where research institutions have the option to contact a volunteer-run organization specializing in the adoption of healthy rats, mice, guinea pigs, and rabbits who are no longer needed in the laboratory [164]. Other places could similarly establish formalized adoption processes to facilitate the adoption of rodents.

Knowing that the research animals we work with now will later become a pet may also result in improved care for the animal; the idea that we are the stewards of someone's future pet places an extra expectation to provide a high standard of care. In addition, this may help motivate specific interventions, including early socialization programs to ensure that animals are comfortable and enjoy interacting with humans.

Even adult rodents who have not been socialized during their time in the laboratory may be suitable candidates for adoption following a short socialization period before adoption. For example, poorly socialized 9-month-old rats were transferred to our laboratory for participation in a short study on playpens. For 3 weeks after our study concluded, we gently handled these rats and provided them with semisolid treats that could not be hoarded (such as pudding or yogurt), thus encouraging them to remain in our proximity. With plenty of continued positive interactions and food rewards, these rats flourished in their adopted homes (Figure 8).

Figure 8: Two Sprague-Dawley female rats who were adopted from a laboratory at the age of 11 months. Left: Clementine receiving head scratches while sitting on her guardian's shoulder. Photo printed with permission from Jessica Ye. Right: Gizmo frequently rests inside her guardian's sleeve. Photo printed with permission from Lexis Ly.

Studying Pets

Work in comparative psychology and animal behavior has long used animals—typically dogs—who live in homes as pets. In 2012, Berns and colleagues published a study in which they trained (using positive reinforcement) 2 pet dogs to sit still inside a functional magnetic resonance imaging machine while a handler signaled whether a food reward was or was not going to be given. The dogs' brains were scanned during the procedure to gather data on reward processing [165]. Since this study, several research groups have imaged the brains of awake, privately owned dogs to gain information on reward, perceptual, social, and communication processing in the brain [166, 167]. Researchers have also used privately owned dogs for behavior studies ranging in topic from how puppies manifest fear behavior [168] to the emergence of jealous behavior [169].

Pets have also participated in biomedical studies using the animal-as-patient model. Instead of inducing diseases in laboratory animals, these studies have used pets who naturally developed the condition of interest, like the use of human patients in clinical trials. Most research in the field of obesity is performed with laboratory rodents, but up to 50% of owned pets suffer from obesity, and they—unlike laboratory animals—also share the genetic diversity, psychosocial stressors, and living environment of humans [170]. Pet rats have a high incidence of tumors, especially mammary gland fibroadenoma [171]. Dogs develop osteosarcoma, and its clinical presentation, biology, treatment, complications, and outcomes are almost identical to those in humans [172]. Many domestic cats develop kidney disease and Type 2 diabetes, and dogs often need hip replacement [173]. Studies of animals with these naturally occurring conditions may prove to be more applicable to humans [174].

There is much scope to build an infrastructure that matches ill pets with relevant clinical trials. For example, the Compar-

ative Oncology Trials Consortium is a network of academic veterinary oncology centers across North America that designs and performs multi-center clinical trials using pet dogs with cancer [175]. A similar infrastructure could be established to include other species and other conditions. The benefits to the "research" animals are clear: when pets are enrolled in a clinical trial, their guardians represent the animals' interests and provide informed consent on their behalf, providing an incentive to prioritize the animals' care and welfare [176]. These "research" animals also lead a normal life as a pet and may actually benefit from the experimental treatment [177].

Conclusion

We have argued that providing laboratory rodents with a good life should be a prerequisite for their use, and we have reviewed research that illustrates a number of approaches to better achieve this. Modest progress could be made in many laboratories immediately and with relatively little effort, such as providing appropriate bedding and shelter, and employing less restrictive handling and restraint techniques (Figure 1). Also relatively short term and with few resources, playpens and socialization protocols can be implemented. Playpens can be built by repurposing cages no longer used for larger species and furnishing them with items from within the facility: empty glove boxes, paper towel rolls, or bins filled with nesting material or shallow water. Socialization protocols can be combined with playpen access; for example, by playfully interacting with animals when transferring them in or out of the playpen. These animals would also become better candidates for adoption.

A longer-term goal is to provide free-ranging options whenever the research goal allows this. Free-ranging animals can have little direct interaction with humans thanks to remote assessment technologies, but if frequent direct interaction is required, these animals (as well as caged laboratory animals) should be trained to voluntarily participate in these interactions.

Our vision for a good life for laboratory rodents includes both excellent living conditions and the opportunity to choose whether to participate in any husbandry and experimental procedure. Although animals can volunteer to receive an injection (eg, in exchange for a food reward), they cannot realistically provide informed consent for an injection (or any other treatment) likely to cause some long-term harm (eg, developing cancer). Laboratories committed to providing a good life may take the stance that they will only perform procedures that the animals choose to experience and that do not cause them long-term harm. This would necessarily preclude work that causes disease. Some projects that would normally cause harm to animals could shift towards the use of pets with naturally occurring injuries or diseases. These animals can be recruited for use in clinical trials, allowing them to potentially benefit from the treatment and under the constraints of informed consent (in this case provided by the animal's caretaker).

We realize that the application of these options will require creative thinking, careful planning, and sometimes substantial monetary and time investment; for example, one important investment will be the allocation of more caretakers, so that these dedicated individuals can afford to spend the time required with each animal. However, we also suggest that these changes can provide a range of benefits, including animals who are more pleasant and interesting to work with, better staff morale, and perhaps data that are more relevant to scientific questions at hand.

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