Mandatory “Enriched” Housing of Laboratory Animals: The Need for Evidence-based Evaluation

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Abstract

Environmental enrichment for laboratory animals has come to be viewed as a potential method for improving animal well-being in addition to its original sense as a paradigm for learning how experience molds the brain. It is suggested that the term housing supplementation better describes the wide range of alterations to laboratory animal housing that has been proposed or investigated. Changes in the environments of animals have important effects on brain structure, physiology, and behavior—including recovery from illness and injury—and on which genes are expressed in various organs. Studies are reviewed that show how the brain and other organs respond to environmental change. These data warrant caution that minor cage supplementation intended for improvement of animal well-being may alter important aspects of an animal’s physiology and development in a manner not easily predicted from available research. Thus, various forms of housing supplementation, although utilized or even preferred by the animals, may not enhance laboratory animal well-being and may be detrimental to the research for which the laboratory animals are used.

Key Words: animal welfare; brain; environmental enrichment; housing supplementation; laboratory animal housing; rodent; well-being

Historical Perspective

The topic of environmental enrichment for laboratory animals evokes, on one hand, amazement at the ability of the mammalian brain to change in response to its surroundings and often, on the other hand, deep concerns for animal welfare. Environmental enrichment was introduced as a research tool for understanding the effects of experience on the brain, whereas attention to the welfare of the animals arose as an emotionally based concern for other living beings. In this article, we argue that animal welfare and environmental enrichment comprise different dimensions of animal housing, and that enrichment does not imply a particular degree of welfare or well being. The relationship between enrichment and well-being, then, is an experimental question or testable hypothesis and not an assumption inherent in the use of the terms. As a result of the research tool and well-being approaches to this field, the rapidly accumulating research from this paradigm has been viewed from these two principal perspectives, both of which emphasize the effects of the housing environment on physiology and behavior but to different ends. In this article, we focus on the potential effects of alterations in laboratory rodent environments on research.

Hebb (1949) first described how increasing the complexity of a laboratory rodent’s environment from a typical laboratory setting improved its subsequent behavior in learning tasks. Hebb brought laboratory rats to his home, where they were treated as family pets. Thus, the first enriched environment was arguably more complex than in any studies published since that time, and surely beyond what any institutional animal care and use committee or biological safety committee would allow today. Subsequently, students of Hebb or others he inspired repeated the basic finding (in the laboratory) that a more stimulating rearing enriched environment enhanced performance on complex learning tasks (e.g., Bingham and Griffiths 1952; Forgays and Read 1962).

Krech, Rosenzweig, and Bennett (1960), who originally found biochemical changes in the brains of rats reared in a complex housing environment and supplemented with daily exposure to novel items in an open field, were the first to coin the term “environmental enrichment” (1962) when describing this paradigm. Since that time, environmental enrichment, variously implemented, has often been used as a research tool to illuminate how the physiology, anatomy, and behavior of an organism adapt to and learn from the environment. Some recent scientific literature illustrates research utilizing environmental enrichment as a research tool: Housing rodents in a complex environment can have effects as varied as the following:

1. Diminishing memory impairment after stroke involving medial cerebral artery occlusion (Dahlvist et al. 2004);
2. Improving behavioral recovery after lesions of the lateral dorsal striatum as well as enhancing the function and morphological development of brain tissue grafts after lesioning (Dobrosy and Dunnett 2004);
3. Inducing neurogenesis in the hippocampus of adult rats (Kempermann et al. 1997; Steiner et al. 2004);
4. Reducing some effects of Huntington’s disease in a mouse model expressing the human transgene for this disorder (Spires et al. 2004);

5. Causing physiological maturation and consolidation of connections in the visual cortex of dark-reared rats (Bartoletti et al. 2004);

6. Increasing “absence” seizures in a rat model (WAG/Rij) of epilepsy (Schridde and van Luijten 2004) but delaying chemically induced convulsive seizures (Auvergne et al. 2002);

7. Reversing deficits induced by prenatal stress, such as decreased social play and increased cortisone secretion, after restraint (Morley-Fletcher et al. 2003);

8. Paradoxically escalating the progression of Alzheimer’s disease in a transgenic mouse model (APP/PS1) of this condition (Jankowsky et al. 2003); and

9. Reversing learning deficits due to a genetic defect in memory (Lee et al. 2003); and

10. Inducing precocious acceleration of visual system development from molecular, physiological, and behavioral lines of evidence (Cancedda et al. 2004).

In general, the foregoing studies used as “enriched” cages enclosures much larger than standard laboratory cages containing a wide variety of structures that allowed for climbing and exploration in three dimensions and in some cases exercise (running wheels), foraging (hidden or scattered food and treats), and hiding or nesting (bedded boxes with a full or partial cover). In most cases, intracage objects were repositioned or exchanged for new ones on a regular schedule to provide a novel environment throughout the exposure period.

**Housing Standards and Supplementation**

Despite the fact that environmental enrichment has been used as a research tool for more than 50 yr, its use as a means of improving husbandry or animal well-being for other than nonhuman primates appears to have been extensively investigated only since the mid- to late 1990s (e.g., Chmiel and Noonan 1996; Sherwin 1996b; Van de Weerd et al. 1997). In these and subsequent studies, environmental enrichment intended to improve animal welfare may refer to any alteration from the minimum requirements of feed, water, and bedding in a standard-sized cage. Despite less stringent expectations for the amount of environmental change, the animal welfare outcome of the change has become a critical focus. Thus, in contrast to a typical definition in research publications of environmental enrichment as a method combining social stimulation with exposure to inanimate objects, generally including some period in a novel environment, a significantly different definition and purpose has been introduced (e.g., Patterson-Kane 2003): The concept now encompasses “an increase in the complexity or naturalness of an enclosure with the goal of improving animal welfare” [italics added]. Olsson and Dahlborn (2002), moreover, argue that the term may be used only after confirmation that an altered environment has enhanced both animal welfare and biological functioning. In these animal welfare-oriented approaches, the types of environmental conditions labeled enriched may be quite dissimilar to previous ones. As one example, Van Loo and colleagues (2002, 2003, 2004a,b) described as “enriched” a Makrolon II (375 cm²) plastic cage with 50 g of sawdust as bedding and two Kleenex tissues. Augustsson and coworkers (2002) and Powell and colleagues (2000) provided this minimum level of supplemental bedding to control cages, whereas an enriched cage included structural items.

Cage size itself is so variable in studies of environmental effects on animal well-being that comparisons involving other factors can be clouded. For example, the standard cage sizes most commonly used for rodent housing in the United States do not match the standard rodent cage sizes in Europe. Moreover, enriched cages for mice alone are available in sizes as varied as the 17 x 22 cm (~.58 in²) Makrolon II cage to enclosures of 15 ¾ x 20 ft (Powell et al. 2000). Because housing density or floor space per animal also affects such things as aggression and stress (Van Loo et al. 2001), the type or level of cage supplementation becomes only one of several environmental variables that may differ among studies.

In addition to altered cage size, “enrichment” for the improvement of animal welfare may refer to increased structural complexity, addition of edible treats, allowance for nesting or exercise, or even as little as a scoop of a specialized bedding that at another institution may be the standard bedding used for all cages. Olsson and Dahlborn (2002) have suggested using more neutral terminology to describe alterations in rodent housing. Because the term enriched has come to have such different meanings—one associated with studies of brain and behavioral plasticity, the other associated with animal welfare—and because the environmental variables studied under the two conditions are very different, we have adopted (and propose) the term housing supplementation as a general description that does not set any expectation with regard to the effects on the animals. Thus the term enriched is used only sparingly in this review.

Exposure to complex environments relative to a standard rodent cage can induce significant, often well-studied, changes in physiology, behavior, and anatomical development. However, when very minor alterations such as supplemental nesting material or a shelter are all that has been added, the possible changes in the animal’s physical status may be more difficult to detect. We discuss below some of the conflicting behavioral and physiological effects of these more practical means of cage supplementation in terms of when they may be beneficial or, in contrast, detrimental to animal well-being or research outcomes.

A conclusion supported by this review as a whole is that we do not understand the mechanisms by which rodents respond physically to environmental changes sufficiently to implement them in a knowledgeable manner. For the pur-
poses of this article, we focus on the most recent studies in which cage supplementation was used in an attempt to improve rodent well-being and subsequently on the effects of substantial enrichment of the laboratory environment on brain and behavior. Olsson and Dahlborn (2002) have conducted an extensive review of the effects of environmental enrichment in mice. The difficulty in comparing such studies and drawing reliable conclusions is amply illustrated by the 14 pages of tables with up to nine columns each that these authors used to portray how one species of laboratory animal is affected by the myriad varieties of cage supplementation currently in use. Changes in strain, sex, age, number of animals per cage, sampling method, and cage size, as well as the type of housing supplementation, can cause changes in physiology and behavior. Furthermore, in contrast to studies using very complex environments as a tool to understanding neural development and function, there has been very little examination to date of neuroanatomical changes that may be induced in animals whose cages have been minimally supplemented with nesting and structural items. We simply do not know whether changes smaller but similar to those we have found in rodents housed in the significantly more enriched environments might be induced by these simpler and more practical types of supplementation.

Animal Welfare Regulations and Environmental Enrichment

Laboratory animal science publications are replete with reviews of the history and current state of regulations pertaining to laboratory animal care and use: the Animal Welfare Act (AWA1); the Public Health Service (PHS1) Policy on Humane Care and Use of Laboratory Animals (PHS Policy1); and subsequent amendments, guidelines, and policies based on those regulations. Many excellent reviews are available (e.g., Siders et al. 1999; VandeBerg et al. 1999) as well as all of Volume 37 Issue 2 of ILAR Journal (Hamm et al. 1995; Nomura 1995; Reid 1995; Townshend and Morton 1995; Wong 1995), which provides background information on laboratory animal guidelines and regulations in the United States and many other countries.

Current US Animal Welfare regulations mandate environmental enrichment only for nonhuman primates. The requirement for exercise of dogs may be satisfied by exercise opportunities outside their pens or runs and might then include increased interaction with humans or conspecifics. However, it is also possible to fulfill the requirement by providing increased living space so that dogs legally may be maintained singly in runs with limited additional stimulation. Currently, the US Department of Agriculture is considering more specific rules for rats, mice, and birds not bred for research. Although the significant changes may affect bird husbandry primarily, more specific rules for captive, wild rodents may have an impact on research results in an important way. Furthermore, we believe it is likely that “laboratory” rats of the genus Rattus and mice of the genus Mus, specifically bred for research, may also be covered soon under the AWA and regulations as well. At that point, US research institutions not already providing cage enrichment for rodents may face AWA requirements for developing “enrichment plans” and policies for additional species (AWA, 9 CFR, 2004).

Whereas institutions governed only by the AWA may not be legally bound to “enrich” the enclosures of animals other than nonhuman primates, research institutions that receive funds from PHS agencies are also governed by the Health Research Extension Act of 1985 and are, thus, under PHS Policy, obligated to meet guidelines described in the National Research Council (NRC1) Guide for the Care and Use of Laboratory Animal Animals (the Guide) (NRC 1996). To comply with the Guide, institutions must consider environmental enrichment for more than nonhuman primates, particularly for social animals that must be housed singly. Although social animals are not listed by species, common laboratory rodents (regardless of sex) clearly are considered social animals and, when possible, should be housed in groups. Moreover, the structural environment inside the cage unit should include items that promote “animal well-being” and allow animals to perform species-typical postures and behaviors. Furthermore, although accreditation by the Association for the Assessment and Accreditation of Laboratory Animal International is voluntary, institutions striving for this designation may adopt site visitors’ suggestions as internal policy, which can easily lead to locally mandated regulations or policies regarding enrichment independent of federal requirements.

Clearly, because most research institutions must provide some means of housing supplementation for the laboratory animals they maintain and use, the effects of environmental stimulation on research and animal well-being are of extreme interest, whether via social housing, structural changes in caging, or supplementation of housing with items intended to promote animal welfare. We review below not only the research on the effects of cage supplementation on animal well-being, but its effects on myriad aspects of animal physiology, neural development, and behavior. In fact, a majority of research in this area indicates that environmental stimulation can have generally positive effects on animal health and well-being compared with an isolated and barren environment. Why then, should animal care providers and administrators not implement immediate alterations to laboratory animal housing for all species? Preference and operant conditioning tests indicate that rodents (Kawakami

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1Abbreviations used in this article: AWA, Animal Welfare Act; EC, enriched cage or condition; Guide, Guide for the Care and Use of Laboratory Animals; IC, impoverished or individual cage condition; ILAR, Institute for Laboratory Animal Research; IVC, individually ventilated cage; NRC, National Research Council; PHS, Public Health Service; PHS Policy, Public Health Service Policy on Humane Care and Use of Laboratory Animals; SC, social condition; SIB, self-injurious behavior.
Supplementation Beyond Regulations

Despite occasional cautions such as those provided by Newberry and the ILAR Committee on Pain and Distress in Laboratory Animals, many recent publications on rodent welfare seem to espouse the view that environmental enrichment is by definition a good thing, without consideration of its possible effects on experimental outcomes or evidence that it is essential for animal well-being. Although isolation housing may induce abnormal behavior in some circumstances (Bayne et al. 1995; Ödberg 1987), the tacit acceptance that group housing is always superior to individual housing is called into question under at least some circumstances (Bartolomucci et al. 2003; Van Loo et al. 2003).
Perhaps more disconcerting than the implication that group housing is essential for all laboratory rodents is the existing assumption that cage supplementation is always better for animals, despite the paucity of data to support this assumption. It appears that animals’ preferences are being allowed to drive if not dictate the issue of what constitutes enrichment. Patterson-Kane (2003), Van Loo and Baumanns (2004), and Van Loo et al. (2002, 2004a) rely on preference testing in an attempt to demonstrate rodent needs for social contact and nesting material. Certainly it is believable that captive mice and rats prefer a cage mate or an opportunity to build nests to a standard cage with little opportunity for activity. When it is possible to provide these attributes and clear that neither experimental results nor animal welfare are compromised in doing so, few researchers would object. The key is in knowing whether experimental results could be compromised, and we propose that in many cases, neither laboratory animal science experts nor researchers can be certain.

Regarding *preference testing* to determine whether cage supplementation is *beneficial* to rodents, it is of value to note that animals’ preferences may not be the ideal guideline to what is of most value to their well-being. In earlier research on addiction, which might not be permitted today, it was found that rats and monkeys given unrestricted or nearly unrestricted access to drugs of abuse (cocaine, amphetamine, methamphetamine, and alcohol) would, within 1 mo, self-administer these drugs to the point of cessation of eating, refusal of hand-fed treats, and in many cases until death, or so near death that researchers removed them from the experiment to provide life-saving measures (e.g., Johnson et al. 1976; Pickens and Thompson 1971). Less dangerous but also of questionable value to animal welfare are the preferences for chocolate and high-sugar, high-fat food common among rodents as well as many humans. This preference and similar findings in other self-selection domains suggest that an animal’s judgment is not always in synchrony with what appears optimal to its health (e.g., Curtis 1985; Galef and Beck 1990).

Despite our misgivings regarding the use of preference testing paradigms to establish enrichment guidelines, particularly in rodents, a strong case can be made for object and housing supplementation in nonhuman primates to prevent or ameliorate self-injurious behavior (SIB) or self-directed biting (see review by ILAR Committee on Well-Being of Nonhuman Primates, NRC 1998). This extreme form of self-directed stereotypy is commonly seen in approximately 5 to 12% of nonhuman primates housed in small single cages with few or no opportunities for exploration or manipulation of objects (Bayne et al. 1995). SIB is extremely rare in rodents under the same housing conditions unless an animal has sustained a sensory or sensorimotor nerve injury that may produce pain, loss of sensation, or paraesthesia. Autotomy of deafferented limbs, especially in association with formation of sensitive neuromas, is often seen in rodents and has been used as a metric in a model of chronic pain (Chudler and Dong 1983; Dong 1989). Although the cause(s) of SIB in nonhuman primates is poorly understood, this maladaptive behavior has been attributed to a “redirection” of targets for aggression, early stressful social experiences (rearing conditions), husbandry practices, research protocol conditions or exposure to multiple moderately stressful events (Lutz et al. 2003; Novak 2003). Novak (2003) found that nonhuman primates have a blunted cortisol response to mild stressors, and monkeys with SIB may have a dysregulation of the hypothalamic-pituitary-adrenal axis. Novak suggests that SIB may be a coping strategy to reduce arousal because the behavior results in a rapid lowering of stress-induced heart rate increases. Alleviation of SIB in nonhuman primates is often achieved by socialization through paired or group housing (Chamove et al. 1984; Weed et al. 2003). However, supplementing the environment by making available manipulative objects (i.e., puzzle feeders) suppressed whole body stereotypy (e.g., pacing and rocking) but not SIB (Novak et al. 1998).

**Effects of Complex ("Enriched") Environment on Brain and Behavior**

As stated above, research on the details of neurological, physiological, and behavioral changes induced by significant environmental alterations was inspired by the work of Rosenzweig and colleagues (see Krech et al. 1960, 1962). An early replication of the Holloway (1966) study using quantitative methods (Volkmar and Greenough 1972) indicated that the dendritic branching of neurons in the rat visual cortex was altered in rats housed in a large group cage with a wide variety of structural items (“enriched cage or condition,” or EC) relative to those housed singly in standard caging (“impoverished or individual cage condition,” or IC) and that “social condition” (SC) rats housed in pairs in standard laboratory cages were intermediate, often differing statistically from both EC and IC rats. The differences between EC and SC rats suggest that even more basic rodent housing alterations, such as social versus individual housing, may actually result in subtle but detectable changes in the brain. The enriched environment used by the Greenough laboratory, although likely falling short of Hebb’s home, is, by contrast, a very complex arrangement of objects for play and exploration, as Figure 1 indicates.

The effects of these environments are not restricted to the brain and to the behavior it enables. Significant peripheral somatic differences exist between rats housed in EC and those in IC that could interact with various sorts of treatments or affect responses to edible reinforcements (Black et al. 1989). In rats in our laboratory, these differences include the following: (1) greater body weight in IC compared with EC rats, accompanied by (2) greater food consumption in the ICs, (3) more rapid maturation of the long bones in IC versus EC rats, (4) sometimes greater adrenal to body weight ratios in EC versus IC rats, (5) a higher kidney to body weight ratio in the EC group, and (6) a lower thymus to body weight ratio in the ECs (with no
The mechanisms mediating the effects described above are largely unknown. Neurotrophic factors such as “brain-derived neurotrophic factor” are known to be altered by environmental variations such as significantly increased cage complexity and exercise (Klintsova et al. 2005; Oliff et al. 1998). These variations may well be a part of the process that generates the alterations in brain physiology and structure in response to altered environmental conditions. This sequence further complicates the stability of the background against which experimental effects are to be measured. Likely related to this, Faherty and colleagues (2005) have recently reported that the drug MPTP (N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine), which kills catechol-

Figure 1 In the first description of an “enriched” environment study, Hebb (1949) reared rats as pets in his home. This laboratory enriched environment, used in the authors’ research, falls short of that environment but, with daily changes of the objects in the cage, provides substantial opportunity for play and exploration.

indication of diminished EC immune-competence; Black et al. 1989). The fact that the organ weight ratios differ in both directions (EC > IC and IC > EC) suggests that the differences do not merely reflect the relatively lower body weights of the ECs.

The goal in reviewing the information above is not to try to explain why these differences occur, but rather to illustrate that a variety of experimental measurements could be affected by differences of this sort. Hence, on the basis of peripheral measures alone, utilizing rats exposed to this type of enriched housing in ongoing experiments could confound results, and certainly switching from nonenriched to enriched animals could generate changes in experimental outcomes. It should also be noted that male and female rats can differ in their responses to enriched environments (e.g., Juraska 1991, 1998). Thus, basic somatic physiological processes are affected by rearing environment complexity, which can affect research outcomes, if those processes are or affect variables of interest. For this important reason, caution is warranted in introducing novel degrees of environment complexity, or enrichment, into ongoing research paradigms.

The brain effects of cage supplementation are even more profound. Neurons and their synapses, vasculature, and the two most prominent types of glial cells are all dramatically affected by exposure to an enriched environment. In visual cortex, the number of synapses per neuron is 20 to 25% greater in EC rats compared with those in IC, with rats socially housed in standard cages typically little different from individually housed rats (Turner and Greenough 1985). This effect is supported by equally substantial increases in the size of the dendritic fields of neurons (Volkmar and Greenough 1972). Synapse morphology and architecture are also different in EC versus IC rats (Faherty et al. 2003; Jones et al. 1997; West and Greenough 1972).

Brain vascularization as assessed by the volume of capillary per neuron is similarly selectively increased in EC rats (Black et al. 1987), presumably in part to “power” the increased number of synapses, a substantial fraction of which are closely associated with mitochondria on the presynaptic side. Astrocytes, which serve to optimize many metabolic functions of neurons, and can be identified both by their structure and by the presence of their characteristic glial fibrillary acidic protein, are increased in both size and number in EC rats (Sirevaag and Greenough 1991). Moreover, synapses in EC rats are covered by fine astrocytic processes more completely than in IC rats (Jones and Greenough 1996). The other macroglial cell type, the oligodendrocyte, which gives rise to the axonal myelination that enhances the speed of nerve impulse conduction, is also affected by environmental enrichment; EC rats have more myelinated axons in the corpus callosum than IC rats (Juraska and Kopcik 1988). All of the foregoing findings have been demonstrated in visual cortex (or connecting corpus callosum), and many effects have also been demonstrated in other brain regions. Taken as a whole, these results indicate that the properties of most cell types and the ways in which they relate to each other in the brain may be altered substantially by the housing environment.

Most of these effects also occur in rats housed in enriched environments for the first time as adults. Most rats used in research are purchased from suppliers as young adults, typically shortly after they reach the point of sexual maturity, and are used as quickly as possible after they have accommodated to their new surroundings, in an effort to minimize cost. If the rats were required to accommodate to an enriched laboratory environment, their bodies and brains might be in a state of relative physiological and structural turbulence at just the time they were expected to be ready to participate in experiments. Clearly research on or involving these variables will be affected, and research on other interacting variables might also be affected in unpredictable ways.

An additional important finding that demonstrates the profound effects of minimal exposure to an enriched environment is the following: Changes in brain produced by experience are manifest to a detectable extent in physiology virtually immediately (Rampone et al. 2000). In brain structure, these changes are detectable in a few days (Wallace et al. 1992).
amine neurons in the substantia nigra in humans (a model of Parkinsonism) and in conventionally housed rats, does not result in similar neural damage if rats are housed in an enriched environment. Although this finding, of course, suggests important therapeutic directions, it also illustrates the complications that might be induced in a well-developed paradigm by the sudden insertion of enriched housing procedures.

Consequences of Supplementation for Animal Well-being: Double-Edged?

Both the NRC Report on Alleviation of Pain and Distress in Laboratory Animals (1992) and the NRC Guide (1996) point out that an adequate environment should allow for the expression of species-appropriate behaviors and should minimize maladaptive behaviors. Thus, small nesting or burrowing rodents such as mice, voles, gerbils, and hamsters may be provided tubes or supplemental nesting material, which they readily shred and mound regardless of whether they are caring for young (Sherwin 1997; Van de Weerd et al. 1997). Rats raised in laboratory cages appear not to use nesting material as readily as mice (in contrast, see Van Loo and Baumans 2004), but are frequently provided a chew toy or shelter (Patterson-Kane 2003). In addition, small rodents may be provided edible treats such as seeds or grain scattered in bedding to allow for a degree of foraging behavior. Such supplements are relatively simple and inexpensive and may allow animal care providers to use their experience with small mammals and their creativity to develop facility enrichment plans (Smith and Hargaden 2001). However, although intuition and even direct observation indicate that such supplementation promotes species-appropriate behaviors, data showing that rodents benefit from these changes are meager and often inconclusive. In fact, rats and mice are notoriously adaptive to a wide variety of environments, both in the wild and in captivity; and whether a standard cage with bedding, feed, and water is insufficient for meeting the behavioral needs of the most common laboratory rodents has not been conclusively demonstrated.

Indeed, recent evidence indicates that certain types of cage supplementation may increase distress in laboratory mice and pose a serious risk to productivity, health, and even life of valuable colony animals. Although preference tests and knowledge of animal behavior indicate that providing shelters in a rodent cage might be valuable, Hae-misch and Gartner (1997), Marashi and colleagues (2003) and Van Loo and coworkers (2002) report that supplementing rodent cages with a shelter increased aggression as well as physiological indicators of stress in male mice. Nesting material such as facial tissues, on the other hand, tended to reduce aggressive behavior as well as stress hormones and adrenal activity (Van Loo et al. 2002, 2003, 2004b). Unfortunately, in more aggressive strains of mice, many of which are very common in biomedical research, fighting is not eliminated by the provision of nesting material, even when soiled nesting material is maintained in the home cage in an attempt to minimize re-establishment of territory (Van Loo et al. 2003, 2004b). Provision of nesting material also can increase or decrease body weight or rate of weight gain, or have no effect, depending on the strain of mouse, as well as alter, in either direction, or have no effect on physiological measures generally considered a measure of stress (Olsson and Dahlborn 2002; Tsai et al. 2002; Van Loo et al. 2002, 2004b). It appears, then, that a practical and often-used type of cage supplementation for rodents that might meet the criterion for allowing species-typical behavior still has variable effects on physiology, including the potential to affect research adversely. For any alteration in the standard cage environment, it is critical to evaluate neuroanatomical changes such as those found in rodents raised in very complex environments in addition to effects on development, parameters of stress, and overt behavior.

Scientists who use animals in research as well as laboratory animal specialists are justifiably concerned about the adequacy of current housing standards. Alterations may affect research as well as budgets and may adversely affect animal well-being rather than enhancing it. Animals that are under chronic stress or engaged in behaviors that either directly compromise their well-being or that may be symptomatic of an abnormal neurophysiological state (e.g., self-mutilation, stereotypies, or targets of excessive aggression) could be unsuitable research subjects. To the extent that a lack of environmental stimulation is responsible for chronic stress or other pathological states or induces maladaptive behaviors, it might be advisable to consider cage supplementation a part of essential animal husbandry.

Some rodents express various stereotypies when they are housed in standard laboratory cages. Bank voles (Cooper et al. 1996; Odberg 1987) and deer mice (Powell et al. 2000), which perform stereotypic behaviors in standard caging, exhibit a decrease in stereotypy when exposed to a more complex enclosure. Based on spatial discrimination studies in bank voles, Garmer and Mason (2002) suggest that stereotypy indicates altered basal ganglia function and argue that conditions that minimize stereotypy (e.g., increased cage complexity and a later weaning age) may enhance research reliability and validity. Powell and colleagues (2000) have shown that increasing the complexity of laboratory caging for deer mice can reduce stereotypy whether provided at a very young age or in older animals once it is established, whereas in bank voles, reducing established stereotypy in older animals is much less successful than when exposure to a complex cage is initiated at a younger age (Cooper et al. 1996). In contrast to the view that stereotypy is a method of coping with stress, Wurbel and Stauffacher (1996) did not find that modifying cages to prevent stereotypic cage-lid bar biting altered measures of chronic stress. In these studies, “therapeutic” caging is not simply a standard-sized cage with nesting material and/or an added climbing structure. Rather, the cages are much larger and more complex than standard cages. There is little evidence that adding individual items such as nesting material or a
structure to a standard cage will ameliorate this condition. In fact, supplementation that might be considered environmental enrichment for laboratory mice (e.g., shreddable material for nesting) is provided in other studies as part of the standard or control environment.

Production is one facet of animal husbandry that might be expected to benefit from cage supplementation. Despite the fact that nesting material and shelters are commonly provided to breeding rodents, few data have been published to indicate how these housing manipulations affect reproductive success. As part of a study investigating effects of individually ventilated cage (IVC) racks, open shelf racks, and ventilated cabinets on the breeding performance of mice, Tsai and colleagues (2003a) tested DBA/2 mice in both standard cages (i.e., bedding only) and cages supplemented with nesting material, a wooden climbing structure, and a small nestbox. Although the breeding index (pups weaned per female) did not differ significantly between enriched and nonenriched breeders in all rack types, overall this level of supplementation appeared to decrease production via increased abortion rates and/or fewer pups born per litter and fewer litters per dam. Furthermore, this type of cage supplementation advanced by several months the age at which production began to wane in breeding females. Although little controlled research has been published on the effects of cage supplementation on reproductive success in the many commonly used mouse strains, research groups with a high stake in maximizing production of valuable mice, especially those housed in the increasingly common IVC racks, should evaluate whether cage enrichment presents an acceptable risk to production.

Effects of Supplementation on Research Results

Considering the vast effects an altered environment can have on physiology, brain structure and function, and behavior, it should be of little surprise that several laboratories have investigated the hypothesis that alterations in environment can affect the validity of research results. Mering and coworkers (2001) have measured various physiological parameters in rats housed under various conditions including supplementation with gnawing blocks and differing group sizes. This group has reported that more animals were needed when examining adrenal gland weights in group-housed animals with gnawing blocks but fewer were needed when looking at group-housed rats without gnaw blocks. Furthermore, certain parameters (weights of brown adipose tissue, epididymal adipose tissue, and adrenals) were more sensitive than others (corticosterone levels, thymus and spleen weight), which in turn were more sensitive than final body weight and growth to this minor form of cage supplementation.

Tsai and colleagues (2003b) also have reported that altered housing effects on behavior can be inconsistent. Using two different “altered housing” systems (E1 and E2), both of equal overall floor size that did not differ from the control cage but one with walls inserts and one with a climbing structure, they documented differences in behavior related to exploration that were due to housing effects between both the control and E1 group and the E1 and E2 groups. Furthermore, females were affected differently than males by the differing housing systems, and the various parameters studied were affected differently, depending on whether the mice had been housed in one enriched cage or another. In a separate study, Tsai and coworkers (2002) provided structures for climbing and nesting as well as nesting material (an environment similar to the E1 system described above) to three different strains of female mice housed four per cage. Although mean blood values (i.e., red and white blood cell counts, hemoglobin and hematocrit values) and mean body weights did not differ between supplemented and standard housing, coefficients of variation were higher for animals in the supplemented cages. The authors suggested that the type of cage supplementation, duration of experiment, sex of animal subjects, and strain all interacted with housing effects. They warned of higher variability, indicating that more animals per experiment might be needed for statistical significance if cages are to be supplemented beyond standard requirements for feed, water, and bedding.

Finally, two studies warrant mention because they indicate the need for caution in introducing various factors. First, the study by Rampon and coworkers (2000) demonstrates how new technologies continue to reveal how much remains unknown in this arena. Given that gene expression is known to be affected by environmental factors, this group used oligonucleotide arrays to investigate gene changes in the brains of mice in response to exposure to large group cages with a wide array of structural objects. Expression of a large number of genes was affected by altering the housing environment. In fact, of 78 genes whose expression was altered at least 1.5 fold, 60 were changed after as little as 3 or 6 hr of exposure to the more complex environment. Many of the genes affected can be linked to neuronal structure, synaptic plasticity, and transmission, a number of which may play important roles in learning and memory.

Second, the results of Crabbe and colleagues (1999) indicate the potential for rapid onset and substantial effects of environmental variations. These authors found that seemingly identical procedures were associated with strikingly different behavioral outcomes in mice in different laboratories. This study again warns us that variability can be very difficult to control between laboratories and that small environmental differences can have significant effects on research results.

Concluding Remarks

Supplementation of standard laboratory cages is fast becoming rule rather than exception in rodent housing, as has been the case much longer for primates. If new rules for the housing and care of laboratory rodents are to be accepted as federal guidelines or law, the rules must be formulated with
great care. We propose using the following points to guide such changes:

1. Animal welfare and environmental enrichment comprise different, not synonymous, dimensions of animal housing, and enrichment does not guarantee improved animal welfare.

2. Animals’ environmental preferences are not a guideline to their well-being and can be physically detrimental.

3. The term housing supplementation may better describe cage additions beyond feed, water, and bedding. This term establishes no expectation with regard to the effects on the animals, and thus can be used to describe an experimental condition when the effects on animal well-being are not yet known or are not the subject of a primary question.

4. In many cases, neither laboratory animal science experts nor researchers can be certain whether supplementing a standard rodent cage compromises animal well-being or research results. When either outcome is in question, environmental enrichment should not be mandated by the institution or oversight agencies.

5. Alterations in housing that clearly promote better health, reproduction, and fitness are valuable to animals and those who use and care for them. However, attempting to improve emotional states that cannot yet be reliably measured may not be valuable for either the animals or the research in which they are used.

6. Variability can be very difficult to control both within and between laboratories. For this reason, it is important not to underestimate the small environmental differences that can have significant effects on research results.

We have previously noted that cage supplementation of certain types can have significant effects on brain development and morphology (Benefiel and Greenough 1998). Research has provided many interesting ideas as to how institutions might improve the standard laboratory environment, but the literature has not revealed how the often precise laboratory conditions required for reliable research can be maintained across significant variations in housing conditions. The study of effects of such variations remains rare, and implementation of environmental enrichment policies rarely takes into account the limited research that has been done. Instead, ideas are often simply pulled from the many recommendations found in laboratory animal science literature. Based on this review, we thus argue that not all—and maybe not most—forms of supplementation are beneficial for laboratory animals or good for research. In fact, although certain species appear to require a more stimulating environment, or at least social contact, to prevent maladaptive behavior or to promote essential (e.g., social, reproductive, and maternal) behaviors, research has yet to provide consistent arguments that all species need improvements in standard laboratory housing, or to make a convincing case for what constitutes appropriate improvement.

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