Potential for Unintended Consequences of Environmental Enrichment for Laboratory Animals and Research Results

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Abstract

Many aspects of the research animal’s housing environment are controlled for quality and/or standardization. Of recent interest is the potential for environmental enrichment to have unexpected consequences such as unintended harm to the animal, or the introduction of variability into a study that may confound the experimental data. The effects of enrichment provided to nonhuman primates, rodents, and rabbits are described to illustrate that the effects can be numerous and may vary by strain and/or species. Examples of parameters measured where no change is detected are also included because this information provides an important counterpoint to studies that demonstrate an effect. In addition, this review of effects and noneffects serves as a reminder that the provision of enrichment should be evaluated in the context of the health of the animal and research goals on a case-by-case basis. It should also be kept in mind that the effects produced by enrichment are similar to those of other components of the animal’s environment. Although it is unlikely that every possible environmental variable can be controlled both within and among research institutions, more detailed disclosure of the living environment of the subject animals in publications will allow for a better comparison of the findings and contribute to the broader knowledge base of the effects of enrichment.

Key Words: enrichment; experimental variable; nonhuman primates; rabbits; rodents

Submission of an animal study proposal (protocol) to the institutional animal care and use committee (IACUC1), in general, requires a rationale for the species selected for the study. Often, the investigator is also asked to indicate whether the research animals need to be excluded from part or all of the environmental enrichment program and if so, the reason. The importance of the first question—the appropriateness of the animal model—is obvious. The investigator’s response to the second question—regarding enrichment—may not receive the same degree of consideration. The researcher may know that the provision of certain environmental enrichments to the animal’s primary enclosure is a standard housing practice at his/her institution; or the researcher may be aware of the numerous publications regarding positive effects of providing enrichment to animals, and wants to provide optimal care for the animals. Adding to the overall inclination to provide research animals with enrichment is the obligation of the IACUC to attend to the regulatory requirement: to promote the psychological well-being of nonhuman primates through an environmental enhancement program; and to implement a behavioral management program that addresses the animals’ structural and social environment, as well as cognitive and physical activity, as recommended in the Guide for the Care and Use of Laboratory Animals (Guide1) (NRC 1996).

Following (or not following) the recommendations of the Guide has implications for compliance with the Public Health Service (PHS1) Policy on Humane Care and Use of Laboratory Animals (OLAW 2002) for institutions that receive PHS funding for research and for accreditation with the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC1) International.

The investigator’s consideration of the best animal model for his/her research should also include factors that have the potential to affect the quality of the animal and the research. For example, it is important to evaluate the quality and amount of the food and water provided. To assist in achieving the research goals, a special diet may be provided to the animals, or the water quality may be specially treated to eliminate certain minerals, adjust the pH, or prevent bacterial contamination. Alternatively, the amount of food or fluid provided to the animals may be restricted for either health or research reasons. Other environmental factors that colony managers and investigators reliably evaluate for impacts on research animals include the following: the temperature, relative humidity, and ventilation conditions of the animal holding room; the quality and schedule of the room’s illumination (e.g., whether a reverse light cycle is important for the animals’ well-being or for the research goals); the presence of extraneous noise in the facility that may negatively affect the animals’ reproductive success or other aspects of their well-being; the use of any chemicals in the animal facility (e.g., in conjunction with the pest control

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1 Abbreviations used in this article: AAALAC International, Association for Assessment and Accreditation of Laboratory Animal Care; BDNF, brain-derived neurotrophic factor; Guide, Guide for the Care and Use of Laboratory Animals; IACUC, institutional animal care and use committee; NK, natural killer; PHS, Public Health Service; SIB, self-injurious behavior; SIV, simian immunodeficiency virus.

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program, or through the use of cleaning agents containing volatile oils as deodorizers); and the quality of the bedding used with some species of animals.

Recently, more attention has been directed at understanding the various effects that enrichment, as one more component of the housing environment, can have on research animals. Depending on the species in question, the concern for the effect of enrichment on the research animal has ranged from inadvertent physical harm to the animal to subtle physiological changes in the animal that may have an impact on the study goals. Environmental enrichment is generally considered to imply an increase in the complexity of the environment in which the animal lives, with the goal of enhancing the animal’s welfare. These changes in the animal’s environment can encompass the variety of food items offered to the animal; whether or not the animal is housed in a bedded cage (i.e., rodents); and additional “structural” enhancements such as nest-building materials, shelves/perches, hiding areas, manipulanda (toys), exercise wheels, climbing/swinging apparatuses, water features, access to the outdoors, and much more. Social enrichment is usually meant to imply conspecific interactions, but in a broader sense can include positive interactions with facility personnel and occasionally contraspecific interactions (e.g., young chimpanzees and dogs). The potential impact of each of these interactions depends on the animal model and the research goals. Ultimately, the decision to include a particular type of enrichment should be based on a consideration of the safety of the animal and staff, whether the enrichment has a demonstrable beneficial effect on the animal, and whether the potential effects of the enrichment are experimentally relevant. However, if one considers the provision of enrichment to be similar to other components of the basic animal husbandry program, the same component can and should be considered for all aspects of the animal’s environment.

The scientific literature is becoming replete with examples of changes in animals (and thus potentially changes in the data they generate) resulting from the increasing use of enrichment with laboratory animals. Indeed, a meta-analysis of the published data has been recommended regarding the potential impact of enrichment on the research animal (Clausing 2004). In part, such an analysis is necessary because conflicting reports have been published regarding the impact of enrichments on animals. Thus, the investigator who is considering whether or not to permit the provision certain of enrichments to his/her animals is faced with sifting through literature that concludes beneficial effects, negative consequences, or no change to the animal. From the perspective of the IACUC, these sometimes conflicting reports are important because they illustrate the potential error of relying exclusively on one published report in making sweeping judgments about animal housing conditions.

The organization of this article is based on a separation of the literature by the principal species for which there is a substantial body of published data regarding the effects of enrichment—namely, nonhuman primates and rodents/rabbits. For each of these species, examples of research are provided that describe physical, physiological, and/or immunological changes; altered susceptibility to disease; changes in brain function or anatomy; or, in some cases, specific behavioral changes in the animals that could influence either the health and well-being of the animal or the research. A broader-based discussion of the behavioral effects of enrichment is outside the scope of this article.

### Nonhuman Primates

#### Physical Harm or Change

One of the most serious potential outcomes to the provision of enrichment is associated with physical harm to the animal. The irony of inadvertent and accidental physical harm coming to a research subject when the intent was to do something beneficial for the animal’s well-being makes this circumstance all the more poignant and regrettable. Unfortunately, not all incidents of harm resulting from enrichments are published, thereby allowing some mistakes to be perpetuated. For this reason, publication of descriptions of the circumstances surrounding negative health consequences directly related to an enrichment device or strategy should be encouraged.

A striking example of animal harm resulting from an enrichment effort is the report by Line and colleagues (1990a) describing the death of the highest ranking female rhesus monkey following the formation of a group of 13 animals, of which 12 were wild caught and thus had prior social experience. Line et al. (1990a) also described other injuries associated with this group formation, including lacerations on the tail, head, and/or hand as well as a fracture of the metacarpals on the hand of one animal. Some animals required hospitalization due to their injuries, others required temporary removal from the home cage for treatment with immediate return to the cage, and a third category of animals required no treatment. One male animal was reportedly severely depressed and was permanently removed from the socialization effort. The group formation was conducted to enhance the social lives of these animals. The authors concluded that the group formation was a success, despite the death of one animal and injuries to most of the other animals in the group. They based this conclusion, in part, on the ethical choice of providing research animals with as much protection from physical harm as possible versus providing them the opportunity to engage in more diverse behaviors.

Changes in the social environment can also lead to self-inflicted injuries, sometimes referred to as self-injurious behavior (SIB). Common manifestations of SIB include hair-pulling and self-biting. The extent of SIB can range from quite mild, when the animal inflicts no damage on its body because the behavior is inhibited (e.g., the animal may touch its mouth to its arm, but not bite or otherwise break the skin), or can produce significant damage, sometimes...
necessitating euthanasia. For example, Chamove and colleagues (1984) demonstrated that macaques who were moved from a social group to individual housing exhibited a higher incidence of self-aggression. The authors concluded that the individual housing precluded social redirection of aggression, and the animals therefore vented their aggression on themselves. Alternatively, Reinhardt (1999) notes that self-biting was completely eliminated in individually housed rhesus monkeys after pair formation. More variable success with the reduction or elimination of self-biting behavior through pair formation has been reported for cynomolgus monkeys. In one instance, SIB was eliminated in five monkeys after pair formation (Line et al. 1990b); however, in another study, similar results were not observed, and no animals evidenced a reduction in SIB (Line et al. 1991). Despite these different findings, it is clear that changes in the social environment can have a profound effect on individual nonhuman primates, whether moving the animal from a social environment to individual housing or the reverse.

Hahn and coworkers (2000) report the occurrence of an intestinal linear foreign body in a cynomolgus monkey after it ingested sisal rope that had been suspended on the outside of the cage for enrichment purposes. Reportedly, the macaques would “groom” the rope, chew on it, and pull on it. In this case, the animal had multiple ulcerations, perforations, and septic peritonitis. The rope was later removed from all of the animals’ cages. Similarly, the provision of long rope or chains has been reported to lead occasionally to the death of a nonhuman primate. Indeed, Mahoney (1992) notes that “facilities must exercise caution when installing such climbing devices as vertically hanging or horizontally suspended ropes and chains . . . because an animal can strangle its neck, limbs, or other body parts . . . “ (p. 27). This caution to ensure animal health and safety as items, intended as enrichments, are introduced into the animal’s environment cannot be overstated.

Other physical changes have been documented as resulting from modifications to the housing environment of macaques. Line and colleagues (1990a) reported an increase in the rate of nail growth of aged rhesus monkeys after formation of a group of animals. In general, the rate of nail growth is reduced in older monkeys (Short et al. 1987). Slow nail growth combined with a low level of natural killer (NK) cell activity in older macaques is correlated with earlier mortality (Coe and Ershler 2001). Line and coworkers (1990a) posed two possible mechanisms for the heightened nail growth rate they observed in the socially housed older macaques: (1) the immune system was activated due to the incidence of wounds sustained by the animals, or (2) the immune status was improved due to the positive changes in the animals’ social and physical environment. Coe and Ershler (2001) have shed some light on the answer to this question, reporting that the highest NK response occurred in old monkeys housed with one other older animal compared with an older monkey housed with a young animal or living alone.

In addition, providing nonhuman primates with extensive space in the primary enclosure can influence the progression of joint disease and mobility. A comparison of free-ranging rhesus monkeys with caged animals of the same age shows that the free-ranging animals have a significantly higher prevalence and severity of degenerative joint disease in the knee and significantly more restricted passive knee joint extension (Kessler et al. 1986). The potential for larger housing conditions to influence studies of osteoarthritis in macaques therefore becomes a consideration.

Physiological Changes

Several parameters have been monitored to assess nonhuman primates for physiological changes following an alteration in their environment that would be considered enriching. One early report documents increases in the level of zinc in rhesus monkeys housed in group cages that were made of galvanized (zinc-coated) wire resulting from the animals chewing on the wire bars (Stevens et al. 1977). However, in some cases no change in the animal’s physiology has been documented. For example, there was no change in the heart rate of rhesus monkeys that were moved into a cage that was 40% larger than their home cage (Line et al. 1989), nor was a change in body weight observed after pair housing compatible female rhesus monkeys (Reinhardt et al. 1988).

Alternatively, statistically significant reductions in heart rate have been documented in individual pigtail macaques that are the recipients of grooming from other animals, although the reduction was not found for animals that initiated grooming or engaged in self-grooming (Boccia et al. 1989). Similarly, allogrooming causes a faster deceleration of the heart rate of individual rhesus monkeys that had recently experienced an increase in heart rate due to a stressful situation, such as the approach of a dominant animal (Aureli et al. 1999). The presence of a familiar, compatible animal has been shown repeatedly to buffer perceived stressors of the subject animal (e.g., Cohen et al. 1992; Epley 1974; Gerber et al. 2002; Gonzalez et al. 1982, 1996; Gust et al. 1994; Stanton et al. 1985). The converse is also true; separation from a familiar animal can result in increases in heart rate and body temperature (Reite et al. 1978), and in some cases this stress is not ameliorated even if the animals maintain visual, olfactory, and auditory contact (Smith and French 1997). Thus, forming pairs or groups of animals, which are later separated for research purposes, may have consequences for the animals that are reflected in physiological changes. The duration of these changes may be relatively brief, but would require assessment for the specific circumstances.

Immunological Changes

Perhaps the most widely researched immunological response measured in nonhuman primates is in association
with concomitant changes in the social environment of the animal. In some cases an animal undergoes social separation, and in other cases the animal is placed into a novel social environment. Specific paradigms evaluated include the separation of infant monkeys from their mothers, peer separation, response to removal from and return to the social group, and response to living with a compatible cage mate. The changes in an animal as a result of social separation are important to a discussion of the effects of enrichment because as animals are socially housed for enrichment purposes, they also may be separated from their cage mate(s) for varying periods of time during the experiment, depending on the research. Often there is a physiological response to these changes, mediated through the hypothalamic-pituitary-adrenal system, that is commonly measured by changes in levels of cortisol (e.g., Laudenslager et al. 1982; Levine 1993; Reinhardt et al. 1989). However, other immunologically based indices of the stress response to social changes include decreases in lymphocyte proliferation in response to mitogen stimulation (Coe 1993; Laudenslager et al. 1985), variability in counts of T cell subsets (Gust et al. 1993), rapid eye movement sleep (Boccia et al. 1989), and reduced levels of the animal’s neutralizing antibody to a bacteriophage (Coe et al. 1987).

Coe (1993) concisely reviewed the influence of psychosocial factors on the immune system of nonhuman primates, and expanded the data by comparing the impact of social enrichment and inanimate enrichment on various immune parameters. Shapiro and colleagues (2000) found that individually housed rhesus monkeys had lower CD4(+):CD8(+) ratios than did either pair housed or group housed rhesus, and group housed monkeys had the highest levels of the cytokines interferon-gamma and interleukin-10. Measures of lymphocyte proliferation in response to various gastrointestinal pathogens and nonspecific mitogens showed a trend \( p = 0.08 \) toward a difference among individually, pair-, or group-housed animals. The authors concluded that the socially housed animals had an enhanced immune response. Shapiro and coworkers (1998) found no effect when they evaluated several immunological parameters resulting from the provision of inanimate enrichment.

Elevations of cortisol in nonhuman primates exposed to new social stimuli have been reported to be more closely related to fear experienced by the animal than as a reflection of aggression exhibited by the animal (Chamove and Bowman 1978). Based on this information, the measurement of cortisol values in monkeys exposed to other common procedures is of interest for placing in context a discussion of the impact of enrichment techniques on the well-being of the animal. To that end, the impact of cage size, cage level, room change, tethering, and sedation on the urinary cortisol response in macaques and baboons has been evaluated (Bentson et al. 2003; Crockett et al. 1993, 2000). To summarize their findings, neither cage size nor cage level (upper or lower tier of cages) produced a significant change in cortisol level (Crockett et al. 1993, 2000). However, ketamine sedation did produce elevations in urinary cortisol in both cynomolgus (male and female) and pigtailed (female) macaques.

Of interest is Bentson and colleagues’ (2003) finding that in male rhesus monkeys, ketamine had no effect on (blood) cortisol level, although Telazol® resulted in reduced cortisol in the morning, but not in the afternoon. The authors also found that baboons exhibited increased cortisol levels after ketamine administration, but no significant increase in cortisol was observed following sedation with Telazol®. Clarke and coworkers (1988) showed similar species variability in corticosteroid measurements of bonnet, cynomolgus, and rhesus macaques after confinement in a transport cage. The rhesus monkeys had the least adrenocortical response to confinement in a transport cage, and the cynomolgus macaque had the highest response. These results underscore the need to assess each species for changes in response to its environment, whether those changes involve routine husbandry procedures or the addition of environmental enrichment. Indeed, the data suggest that not many aspects of the primate’s environment are controlled, and that the effects of enrichment provision do not differ significantly from other animal care and use procedures.

Response to Disease and Other Conditions

In general, the response of nonhuman primates, principally macaques, to the progression of a disease or other condition, or the response to disease challenge, is studied under altered social environment conditions. In many cases, a monkey is removed from its social environment either intermittently during the experiment or for the duration of the experiment. Vulnerability to addiction has also been assessed in these types of studies. Juvenile rhesus monkeys have been shown to consume more alcohol when intermittently separated than when they were socially housed, and the intermittently separated monkeys drank more alcohol than continuously separated monkeys (Kraemer and McKinney 1985). Individual differences were also noted; some monkeys were more likely to drink larger quantities of alcohol than other animals. The authors concluded from these findings that social stressors can elicit alcohol abuse and addiction in primates.

Social housing has also been found to have an impact on cocaine addiction. A relationship has been postulated between drug addiction and monoamine concentrations in the brain. Measurements of dopaminergic function in individually and socially housed cynomolgus monkeys via positron emission tomography revealed no differences in individually housed monkeys, but higher levels or more available dopamine D2 receptors were detected in dominant monkeys housed socially compared with subordinate animals (Morgan et al. 2002). The following observations were then linked to these findings: (1) cocaine served as a reinforcer for subordinate, but not dominant, animals (Morgan et al. 2002); and (2) animals living in chronically unstable social groups, wherein group membership changed on multiple
occasions over a protracted period of time, had lower concentrations of serotonin and monoamine metabolites in the prefrontal cortex (Fontenot et al. 1995).

The implications of the studies cited above are numerous. The definition for a “social group” is not entirely clear. It is possible that the social relationships among individually housed animals that have been maintained in the same room for a long period of time could be well established, to the point where they function to some degree as a social group. In such a case, transfer of animals in or out of the room on a routine basis could have an impact similar to that described for socially housed animals that are in physical contact. Similarly, moving an animal from a social group to individual housing for the purpose of an experiment, or putting a previously singly housed animal in a social group, could have an influence on addiction studies.

Several studies evaluating the social environment of a nonhuman primate and that animal’s susceptibility to disease have demonstrated differences between animals maintained in individual housing and group housing, as well as differences between dominant and subordinate individuals within a group. An evaluation of cardiovascular disease in animals experiencing social changes has shown that chronic individual housing of female cynomolgus monkeys augments the incidence of atherosclerosis (Watson et al. 1998). In group-housed cynomolgus monkeys, atherosclerosis is more prominent in dominant males housed in unstable social groups (although not when the group membership is held stable) and subordinate females, regardless of the stability of the group membership (Kaplan and Manuck 1999).

More consistently, rhesus monkeys living in an unstable social group that exhibited more agonism and less affiliative behavior have been reported to have a reduced survival rate after infection with simian immunodeficiency virus (SIV) compared with animals living in a stable social group (Capitanio et al. 1998). Interestingly, rhesus monkeys that engaged in affiliative social interactions had lower plasma concentrations of the virus whereas those animals receiving more social threats had higher concentrations. A detailed review of animal records that include data regarding animal housing conditions and SIV inoculation date has shown that social disruptions (e.g., housing relocation, social separation, or placing into a social group) occurring in close temporal juxtaposition to the inoculation result in reduced survivability of the animals (Capitanio and Lerche 1998). In addition, although there was no effect on the antibody response of male rhesus monkeys related to the timing of a tetanus vaccination and social disruption (placement into individual housing), the behavioral dimension of “high sociability” and “low sociability” expressed by different animals was associated with the strength of the antibody response, with the high-sociable animals having a significantly higher antibody response (Maninger et al. 2003).

The role of inanimate environmental enrichments in causing disease in nonhuman primates has been evaluated only preliminarily. Colleagues and I (1993) have documented that microbial growth persisted on a type of enrichment toy even after sanitation in a mechanical cagewasher. Because the bacterial contamination was limited principally to bacteria commonly found in the environment (Bacillus, Staphylococcus, Micrococcus, nonenterics, and mixed coliforms), we concluded that the potential for toys to cause disease is low. However, the possibility of this contamination confounding infectious disease work should be considered.

**Rodents and Rabbits**

**Physical Harm**

The most significant unexpected impact occurs when an item intended to enhance the animal’s welfare results in harming the animal in some way. Although not many examples of such an occurrence have been reported in the literature for rodents and rabbits, there are two instances of note. Shomer and coworkers (2001) described an injury to a New Zealand White rabbit from a whiffle ball (a hollow plastic ball with numerous holes in its surface) provided to the rabbit for play activity. Although the whiffle ball was considered by facility staff to be too hard to be broken by the rabbits, and too large to be swallowed, a ball unexpectedly became entrapped in the incisors of one rabbit. The outcome of this event was that the animal could not eat or drink for about 12 hr, and there was trauma to the gums. Athymic nude mice have reportedly been adversely affected by cotton nesting material (Nestlets®) placed in their cages (Bazille et al. 2001). In this case, all animals provided with this nesting material manifested conjunctivitis within 2 wk of exposure. In some animals, portions of the nesting material were trapped in the conjunctival sac. The severity of the condition continued to increase until the cotton material was removed. The authors suggested that this finding was limited to athymic nude mice due to the absence of eyelashes in these animals that would otherwise help to exclude particulate material derived from the Nestlets®. Clearly, these cases represent a minority of incidences, but they nevertheless illustrate the point that implementation of environmental enrichment should include an assessment of the potential risk to the animal associated with the item.

**Physical Changes**

Since the early 1950s, investigators have documented physical changes in rodents resulting from changes to their environment. These studies initially focused on how the handling of rats (also referred to as gentling) resulted in a significantly higher average weight gain for the handled rodents compared with the nonhandled, control animals (Bernstein 1952; Weininger 1956; Weininger et al. 1954). This greater weight gain among handled rats was consistent whether the animals were individually or group housed (Ruegamer et al. 1954). This increase in weight was later shown to be related to greater amounts of fecal pellets ex-
creted by the control animals, with a presumed increased “food utilization” by the handled rats because the amount of food consumed did not differ significantly between handled and unhandled rats (Ruegamer et al. 1954). Handled animals also tended to have a greater skeletal length than control animals (Ruegamer and Silverman 1956).

The finding of weight gain in rodents exposed to a more complex environment has been extended to include influences from nonsocial enrichments. For example, Augustsson and colleagues (2003) found that BALB/c and C57BL/6 mice housed in enriched or “superenriched” cages gained more weight than control mice (nonenriched cages), even though food consumption did not differ significantly among the groups. However, such findings appear to be inconsistent among laboratories evaluating the same strains of mice. Van de Weerd et al. (2002) found that mice reared in superenriched and enriched conditions gained weight faster than mice (RIVM:N:NIH, BALB/c) housed in nonenriched cages, but the superenriched mice also consumed more food than mice housed in the other conditions. Tsai and coworkers (2002) reported that BALB/c, C57BL/6, and A/J mice living in enriched (nest box, nesting material, climbing bar) or nonenriched cages had mean body weights that either were not different or differed only slightly from each other (heavier or lighter), but not to a statistically significant degree. Similarly, Smith and colleagues (2000) evaluated the body weights of CD-1 mice provided with a synthetic gauze pad as enrichment and found no difference in either body weight or weight gain from that of control animals (no gauze pad).

Researchers have also compared the body weights of New Zealand White rabbits living in enriched and nonenriched environments. Harris and coworkers (2001) evaluated enrichments that included food items (celery, Bunny Blocks®, Bunny Stix®) as well as nonfood items (Jingle Ball®, Kong® toy, Nylabone®). Not surprisingly, the food-enriched rabbits showed weight gains, although not to a statistically significant degree after 15 days, compared with rabbits receiving only nonfood enrichments.

Organ weights have also been measured for an influence from environmental enrichment. Tsai et al. (2002) weighed the heart, liver, kidney, adrenal, spleen, and uterus of three inbred strains of mice (BALB/c, C57BL/6, A/J) living in enriched and nonenriched cages. In comparisons with control animals, they found that the weights of the spleen from enriched animals were slightly, but not significantly, increased; and the weights of the adrenal from enriched animals were slightly, but not significantly, decreased. This latter finding is further supported by Bonnet and coworkers (2004), who have documented statistically significant lower adrenal weights in female B6C3F1 mice housed in stainless-steel cages with a mat of hemp fibers compared with animals housed in cages without a mat. They also found that the thymus weight of mice housed in social groups (in plastic cages) was higher than individually housed mice in stainless-steel cages. Smith et al. (2000) found no significant differences in the organ weights (kidneys, liver, heart, spleen, testes, prostate, adrenals, thyroid and parathyroids, pituitary, and brain) of CD-1 mice with a gauze pad in the cage compared with mice without a pad.

Numerous studies have also evaluated the effect of environmental enrichment on recovery from an experimentally induced physical change (e.g., injury) in the animal. A very early study (Hammett 1921) showed that the relative mortality subsequent to thyroparathyroidectomy and parathyroidectomy of rats was much lower (13%) in animals that had been handled compared with animals that had not been handled (79%). In more recent examples, rats with experimentally induced traumatic brain injury living in an enriched environment exhibit a shorter latency to find the platform in a Morris Water Maze test compared with injured individually housed rats (Hamm et al. 1996, Passineau et al. 2001). This finding indicates facilitated recovery of some cognitive functions from exposure to enrichment items. Of note is the report that the brain injury in the enriched rats was approximately two times smaller than the individually housed rats after 2 wk (Passineau et al. 2001). Rats having undergone ligation of the middle cerebral artery and living in an enriched environment had significantly better motor function and improved more quickly than control animals (Grabowski et al. 1995; Johansson 1996; Ohlsson and Johansson 1995), even when the enrichment was limited to social housing (no inanimate enrichment) compared with individual housing (Risedal et al. 2002). Brain injury induced by a septal lesion in mice living in enriched or nonenriched environments was also influenced by environmental complexity.

In rodents, a septal lesion produces a phenomenon known as septal rage, which is characterized by hyperemotionality and aggressiveness by the rodents. Enriched mice were less “reactive” than nonenriched mice, and mice living first in an enriched environment and later transferred to the nonenriched environment showed an immediate increase in reactivity (Goodlett et al. 1982). In addition, the amount of exposure to enrichment may not need to be very long to be effective. For example, Will and coworkers (1977) noted that rats that received 2 hr/day of enrichment recovered from bilateral lesions to the occipital cortex to the same degree as rats exposed to enrichment 24 hr/day.

**Neurological Changes**

Morphological changes to the brain are perhaps among the most well-known effect of enrichment on rodents. Walsh (1981) has reviewed the effects on the brain in response to environmental complexity. Typically, greater cerebral weight and length, as well as increased cortical depth, are measured in rodents living in an enriched environment (e.g., Diamond et al. 1976, 1985). Recently, the specific regions of the rodent brain affected by living in an enriched environment have been determined. In the hippocampus, CA1 and dentate gyrus cells were affected; however, changes were not observed in layer V pyramidal neurons of the
cerebral cortex or in the spiny neurons in the striatum (Faherty et al. 2003). When the histological changes in the brains of rats provided with social and inanimate enrichments have been compared with rats that are socially housed without inanimate enrichments, the number of oligodendrocytes and astrocytes in the occipital cortex have reflected an increase in the former group. With rats that were handled daily, only the number of astrocytes increased (Szeligo and Leblond 1977).

Increases in dendritic spines and branching, synaptic connections, and neural cell size have long been recognized to result from enriching the environment of rodents (e.g., Globus et al. 1973; Volkmar and Greenough 1972). These effects on brain morphology cannot be attributed to the enriching effects of social housing, but rather appear to be related to the presence of the inanimate enrichments (Renner and Rosenzweig 1986, Rosenzweig et al. 1978) and, more specifically, to direct contact with the inanimate enrichments (Ferchmin and Bennett 1975). Although enriched mice move longer distances and spend more time in the center of an open field testing apparatus (Kohl et al. 2002), it appears that the increased activity associated with exploring and manipulating inanimate enrichment items is not responsible for the histological changes in the brain (Faherty et al. 2003; Kohl et al. 2002).

However, an examination of gene expression in the brain, relative to the availability of enrichment items in the home cage, reveals that enrichment affects the expression of several genes that regulate neuronal structure, synaptic signaling, and brain plasticity (Rampon et al. 2000). In fact, the transgenic R6/1 and R6/2 mice, which are used to model Huntington’s disease—a genetic disorder that results in motor dysfunction, dementia, and death—exhibit less decline in select motor function tasks and delayed loss of cerebral volume in those transgenic mice living in an environment that includes both social and inanimate enrichment (Glass et al. 2004; Hockly et al. 2002). Recent evidence suggests that the mode of action for enrichment on slowing the course of the disease is mediated through reducing protein deficits in the brain (Spires et al. 2004), because nonenriched mice have significant decreases in brain-derived neurotrophic factor (BDNF) as well as reductions in dopamine levels. Not only is BDNF higher in the brains of enriched animals, but so too are nerve growth factor and neurophin-3 proteins (Ickes et al. 2000). Hockly and colleagues (2002) note that standardization of the housing condition should be considered in therapeutic trials. However, simply using R6/2 mice in behavioral testing (rather than housing them in enriched cages) also improves their survivability (Carter et al. 2000). So, it appears that there are a number of potential confounding variables, some of which may not have even been identified to date, that can alter the course of experimentally induced or modeled disease research.

A discussion of the possible neurological changes would not be complete without mentioning the effects of enrichment on memory and learning because these effects reflect the functional change that can occur in rodent brains concomitant with the anatomical changes already described. Learning rate has been described to be faster in enriched rats than nonenriched rats (Kobayashi et al. 2002; Wainwright et al. 1993). Additionally, spatial memory, in general, is positively affected by an enriched environment (e.g., Martínez-Cue et al. 2002; Nilsson et al. 1999), even when the enrichment is provided to the animals when they are adults (Frick et al. 2003). This effect has also been documented in rats with induced status epilepticus (Faverjon et al. 2002). Although the precise mechanism of the memory enhancement has not been identified fully, recent evidence suggests that enrichment affects cAMP-dependent protein kinase long-term potentiation in the hippocampus (Duffy et al. 2001).

**Physiological Changes**

The cardiovascular system and hematology of rodents from enriched and nonenriched environments have also been assessed. Tsai and coworkers (2002) found a nonsignificant decrease in red blood cell count and hematocrit and a nonsignificant increase in hemoglobin in enriched mice (BALB/c, C57BL/6, A/J) compared with nonenriched controls. They also observed a nonsignificant increase in the level of white blood cells in enriched C57BL/6 and A/J mice, but not in enriched BALB/c mice. Similarly, no effect on the hematological profile was observed in New Zealand White rabbits provided with a stainless steel rattle clipped to the cage (Johnson et al. 2003). Social enrichment alone can result in an increase in systolic blood pressure (but not diastolic pressure or heart rate), and nonsocial enrichment in combination with brief periods of social enrichment (2 hr/day) led to increased systolic and diastolic blood pressures but no change in heart rate. When rats that were previously socially housed were changed to individual housing in enriched cages, all three dimensions measured (systolic blood pressure, diastolic blood pressure, and heart rate) increased (Lawson et al. 2000). However, even routine procedures such as cage changing can lead to significant, albeit brief (45- to 60-min), increases in systolic and diastolic blood pressure as well as heart rate (Duke et al. 2001).

The effect of enrichment in the environment has been measured for several other physiological parameters. Higher levels of testosterone and immunoglobulin G levels have been detected in enriched mice compared with control animals, although there is some strain variability in these findings (Nevison et al. 1999). Plasma triglyceride levels have been reported to be lower in individually housed rats, compared with socially housed rats, although individual housing did not result in a concomitant increase in total cholesterol levels (Pérez et al. 1997). Lower cholesterol values were noted, however, in rats housed in large, enriched cages compared with rats housed in standard plastic cages (Augustsson et al. 2002). The urine corticosterone...
level of these “pen-housed” rats was higher than that of the rats housed in standard cages, which the authors attributed to the greater activity exhibited by the rats in the large pens. Conversely, the plasma corticosterone of rats in response to stress (maternal separation) was lower for enriched animals than for nonenriched animals (Francis et al. 2002), but no difference in corticosterone (or thyroxine) levels was observed in enriched versus nonenriched DBA/2 mice (Tsai et al. 2003).

Enrichment items are not the only environmental factors that have the potential to influence the physiology of a rodent. Cage size alone is a potentially confounding factor for some types of research. Specifically, Kühnen (1999) found that the basal rectal temperature of hamsters housed in smaller cages was significantly higher than that of hamsters housed in larger cages, and the animals’ response to a fever-inducing lipopolysaccharide also varied depending on the cage size. The depth and type of bedding placed in the cage also influence body temperature (Gordon 2004). Mice housed in deep wood bedding were noted to have a significantly higher temperature than comparable mice housed on a layer of beta chips or thin wood bedding, although this difference was time dependent because it was observed only during the daylight hours. As Gordon (2004) notes, such a difference in body temperature based on bedding depth and type would be of concern in toxicological studies in which determination of the endpoint is based in part on the animal’s body temperature.

Discussion

Several key points emerge from this review of the potential effects of environmental enrichment that bear further discussion. First, there is no consensus regarding what constitutes enrichment for some animal species. For example, many facility personnel do not consider solid-bottom, caged cages a form of enrichment. However, some researchers do believe that this type of cage constitutes enrichment because rodents, for example, can burrow into it, thereby expressing a species-specific behavior. However, bedding also can affect the research animals. Indeed, contact bedding (Shred-N-Nest®, a corn-husk product) has been shown to reduce the aggression exhibited by male BALB/cAnNHSd mice (Armstrong et al. 1998), although the aggression among male BALB/cAnNCRLBr mice was not attenuated by the provision of Lignocel® 1/4 sawdust bedding alone (Van Loo et al. 2002).

Bedding can also pose a problem for rats dosed with buprenorphine, for example after surgery, due to the quantity ingested as a result of pica behavior (Clark et al. 1997). Some types of bedding (e.g., pine, cedar) emit volatile hydrocarbons, which can interfere with the induction of cytochrome P450 enzymes in hepatic microsomes (Vesell et al. 1973), while others can modify the mucosal immune response, as evidenced by increased numbers of Peyer’s patches in the intestinal mucosa (Sanford et al. 2002). Weichbrod and colleagues (1986) have reviewed many of the effects of different bedding materials on laboratory animals.

Similarly, some studies refer to the provision of increased cage space as a form of enrichment with positive results for the animals (Augustsson et al. 2002), although the provision of increased cage size alone has been found to promote the expression of undesirable behaviors by some species of animals (Bayne and McCully 1989). Thus, although the type of bedding used and the cage size provided the animals may be standardized in an animal facility, this very standardization may result in unexpected or undetected confounding variables in the research.

A second key point is that a number of factors in the animal’s proximate environment can affect the animal and the research data. Some environmental variables of importance include the quality and quantity of illumination in the animal room (e.g., Lanum 1979; Novak and Drewsen 1989), sound (Kaplan and Miezejeski 1972; Novak and Drewsen 1989), food and water quality, air quality, temperature, how much the animals (especially rodents) are handled, and even the demeanor of the people interacting with the animal. So, of the innumerable environmental variables that can have an impact on the animal research model, enrichment techniques are simply a part of a larger picture of the overall housing environment that should be considered.

Characterizing a particular feature in the research animal’s environment as an enrichment is an artificial delineation that perhaps should be abandoned. Rather, it is more appropriate to assess the gestalt of the animal’s environment. Questions that should be addressed include whether the animal’s well-being is enhanced by including a particular feature in the environment, whether a better animal model results, or whether increased variability is introduced into the study. If the latter, then a second tier of decision-making should occur because different aspects of the principles of refinement, reduction, and replacement (“the 3 Rs”) are involved. In providing certain elements such as enrichments, to the animal’s environment, a refinement is introduced. However, if the enrichment also causes more variability in the study, if more animal subjects are needed to achieve appropriate statistical power, or if studies must be duplicated, then the goal of using fewer animals is not achieved. Investigators and IACUC members need to balance the issues of enhanced animal welfare with the potential for reduced animal numbers used in the research.

In some cases, the very finding that an enrichment technique has modified the response of an animal to a disease, addiction, or condition has proven to be of interest (Gordon 2004; Hockly et al. 2002; Kühnen 1999). This result suggests that preventing or delaying the onset of clinical signs, or possibly even reversing some signs, as a result of living in an enriched environment (Francis et al. 2002; Ohlsson and Johansson 1995; Passineau et al. 2001) may have implications for the human condition (Glass et al. 2004; Hockly et al. 2002; Kobayashi et al. 2002).
Concluding Remarks

As illustrated by the preceding review, the effects of environmental enrichment are similar to those of other environmental components. In some cases, there is no impact on the animal or research parameter being studied, and in other cases there may be an effect on either the animal or the research data that might be construed as either positive or negative, depending upon the specific circumstances. Although it is unlikely that every possible variable can be controlled both within and among research institutions, more detailed disclosure in journals and other publications of the living environment of the subject animals in publications will allow for a better comparison of the findings, and contribute to the broader knowledge base of the effects of enrichment.

References


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