Preparation of Animals for Use in the Laboratory: Issues and Challenges for the Institutional Animal Care and Use Committee (IACUC)

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Abstract

Preparation of animals is important for optimization of animal welfare as well as to minimize interanimal variation, thereby strengthening the quality of data for in vivo studies. These issues are important in the work of institutional animal care and use committees (IACUCs), but they pose many challenges as well. This article provides IACUC members with a resource for use in determining whether and how preparation of animals for research affects the IACUC's responsibilities. The topics addressed are intended to serve as a starting point for consideration and discussion. Questions related to subject selection and acclimation of subjects to experimental housing and procedures are emphasized and should provide IACUC members with a framework for discussion of relevant questions. Guidelines are provided for promoting the acclimation of a number of species to experimental settings. Additional, potentially controversial points are also raised, including the effects on longitudinal data sets of changing subject preparation procedures. The roles of the IACUC in the research endeavor are multifaceted and continuously evolving. As empirical data are produced that affect additional aspects of animal care and use, it is important for these committees to be able to evaluate and, when appropriate, stimulate the implementation of improved procedures and strategies.

Key Words: acclimation; desensitization; habituation; homeostasis; IACUC; subject selection; variability

Introduction

This final article of the current issue of *ILAR Journal* is specifically aimed at providing members of institutional animal care and use committees (IACUCs[1]) with a basic summary of some of the information contained in the issue. The topic "Preparing Animals for the Laboratory" is of particular importance to IACUC members as they interface with and form a bridge between the institutional animal care and use program and the research program of principal investigators whose protocols undergo IACUC review. Information regarding whether and/or how well animal models have been prepared for research requires the interaction of everyone in the animal production and utilization chain, and the IACUC has an important role to play.

IACUC members are interested in (1) how animals are prepared for residence in the institutional animal care facilities, (2) the associated program of attendant animal care, and (3) how animals are prepared for proposed experimentation. To date, there has been relatively little published information concerning these topics, and relevant data that do exist are not always easily accessed from the biological and behavioral literature. The articles in this issue are intended to provide interested readers, including IACUC members, with valuable information, relevant guidelines, and, most importantly, *data* to help them facilitate the performance of their roles in the research endeavor.

Certain aspects of the preparation of animals for use in the laboratory are derived and conducted primarily on the basis of good common sense, experience, and competence of the involved professionals. These aspects may include processes related to assuring that (1) high-quality animal models are obtained from appropriate vendors; (2) vendors attempt to provide animals that require a minimum of special or remedial preparation; and (3) transportation, receipt, and acclimation of animals are accomplished with as few perturbations as possible. To a great extent, many of these aspects of animal preparation are handled by the animal care/animal resources/veterinary group prior to, and usually without, the direct involvement of the IACUC. Nonetheless, the IACUC is responsible for oversight of the program of animal care and use through its semiannual inspections and program reviews, and IACUC members should be cognizant of animal preparation procedures.

The IACUC can access and evaluate animal acquisition and experimental preparation in a number of ways. For example, the IACUC relies on the vigilance of the animal resources group to make certain that among other things, appropriate procedures for health assessment and health monitoring are in place. These procedures are often formalized as standard operating procedures (SOPs[1]), especially in a good laboratory practice environment. Topics related to acquisition, transport, and receipt, which are covered in considerable detail in the articles in this issue by Conour and

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[1]Abbreviations used in this article: 3Rs, replacement, refinement, reduction; IACUC, institutional animal care and use committee; SOP, standard operating procedure.

colleagues (2006), Meunier (2006), Obernier and Baldwin (2006), and Smith and Swindle (2006), should be a convenient resource for evaluating existing procedures and/or creating policies.

As noted, the semiannual IACUC inspection provides an ideal forum for program review, when the IACUC can be apprised of relevant information concerning vendors, transporters, and receipt and acclimation procedures. During these program reviews, IACUC members—including members of the animal resources group—have the opportunity to question procedures, present relevant new findings from the literature or their own work, and suggest modifications to animal management practices. When IACUC members have knowledge concerning the underlying literature-based rationale for institutional SOPs, they are in an excellent position to make judgments or improvements, and to evaluate standard practices.

Many of the points emphasized in this article focus on two major issues: the *selection* of the most appropriate subjects for research projects, and the *acclimation* of the subjects to the conditions they are likely to experience while in the laboratory and in research projects (Table 1). For many of the nonrodent species, especially those that are likely to spend extended periods in the laboratory, knowledge of the behavior of the animals in the wild and management of their behavior in captivity are essential for proper preparation and the provision of high-quality care (Bloomsmith et al. 2006; Capitanio et al. 2006; Scipioni Ball 2006; Smith and Swindle 2006; Tardif et al. 2006). Other critically important concepts addressed in this article are variability, homeostasis, and equilibrium.

Table 1 (A) Potential criteria for subject selection and (B) potential factors that require acclimation

A. Criteria for subject selection	Examples
Age	Young animals only
Sex	Male only
Species/strain	Sinclair pigs; knockout mice
Experimental history	Naive animals; hepatitis C carriers
Social experience	Mother-reared; nursery-reared
Training experience	Sling-trained; voluntary blood samples
Availability	Surplus adult males
B. Factors requiring acclimation	**Examples**
Physical housing conditions	Small cages; indoor light cycles
Social housing conditions	Single housing; same-sex pairs
Dietary	Experimental diet; enrichment food
Experimental apparatus	Slings and other restraint devices
Handling procedures	Capture; gavage; sampling

Although it is typically stated that scientific research should be conducted on animal models that are as normal as possible, this intent is not necessarily consistent with research practice. Normality, both in the physiological as well as the behavioral sense, must be placed in the context of the laboratory and the experimental situation. For example, although inbred and genetically modified strains of rodents are not necessarily normal with respect to wild-type animals, investigators attempt to achieve experimental systems that are not unduly affected by uncontrolled variation. Research on these "non-normal" subjects can be valid if (1) proper experimental designs are used, and (2) animals are not housed or handled in ways that compromise their welfare or either directly or differentially affect research results.

Preparation of Subjects and Replacement, Refinement, and Reduction (3Rs[1])

IACUCs are typically cognizant of the 3Rs (Russell and Burch 1959) and, more specifically, are typically interested in assessing whether research protocols adequately address these objectives. They are specifically charged during protocol review with assuring that investigators have searched the literature and have provided justification that there are no "alternatives" for the proposed use of animals, or that they have incorporated such information into the experimental plan. It is difficult for IACUC members to assure that investigators have "refined" their methods, although most committees strongly endorse these endeavors and encourage investigators to share this information with the IACUC within their experimental descriptions on the protocol review form. In fact, in Sweden, ethics committees (the European counterpart to the IACUC) have had a measurable effect on the implementation of several refinements to experimental procedures (Hagelin et al. 2003; Hau et al. 2001).

Although replication is critical to the scientific process, duplication of research effort is not, and IACUCs are charged with reviewing this aspect of proposed animal use. One of the important ways in which investigators can refine protocols and properly replicate previous work is by adopting methods that diminish interanimal variability in experiments through standardization of methodology as well as proper selection and preparation of high-quality animals.

Procedures that decrease random variability between subjects—a critically important goal of most preparation procedures—also decrease the number of subjects required for experiments to achieve satisfactory statistical power (a term that is discussed in more detail below). Refining procedures that decrease stress for experimental subjects can

improve the welfare and care of the subjects and can potentially decrease variability simultaneously. Few data exist to address this point, and some might argue that increased stress decreases variability. However, obtaining voluntary blood samples from rhesus monkeys and chimpanzees is an example of an experimental innovation that is a refinement, which could eventually result in a reduction in the number of subjects required for certain experimental assessments (Pranger et al. 2006; Schapiro et al. unpublished data).

Optimizing Animal Models

The ultimate goal of all providers and users of animals in biomedical research is to obtain valid and reliable data by optimizing the quality and utility of their animal models. By developing and using the best available models, research can be performed that is minimally affected by confounding factors. Proper preparation of experimental subjects should minimize these variables and factors in experimental designs, thereby yielding high-quality data that *directly* address experimental hypotheses.

Most IACUCs focus on how care and handling affect welfare and whether the numbers of animals used in experiments are appropriate. The information contained in the articles in this issue confirms that preparation and conditioning procedures are likely to have an impact on the data. This fact strongly supports the notion that the IACUC review of animal preparatory processes should evaluate procedures with a "balanced view" of both animal welfare issues and the data needs of the experiment. While it is often stated, usually accurately, that good welfare goes hand in hand with good science, there are many instances in which IACUC members must balance and analyze multifaceted costs and benefits of specific procedures. The recognition and control of stressors and the knowledge of adaptation to husbandry and experimental conditions are often instrumental in the understanding of study outcomes (Weihe 1998).

Subject Selection

Several of the articles in this issue emphasize the critical role that the selection of subjects plays in laboratory research (Bloomsmith et al. 2006; Capitanio et al. 2006; Smith and Swindle 2006; Tardif et al. 2006). Factors that influence the selection of subjects factors that are dependent on the research question (Sinclair pigs for malignant melanoma research [Smith and Swindle 2006]; chimpanzees for studies of certain human monoclonal antibodies [Bloomsmith et al. 2006]) and other outside factors that are dependent on the characteristics of the animal model or its availability (male ferrets to avoid estrus issues [Scipioni Ball 2006]; excess juvenile males in nonhuman primate breeding colonies [Capitanio et al. 2006]).

Variability

The role of the IACUC has typically been to ensure appropriate care and use of animals in research. Currently, in some research institutions, there appears to be an increasing emphasis on evaluations of the statistical adequacy of research designs as more and more biostatisticians are increasing their involvement with IACUCs. One aspect of this statistical scrutiny is increased attention to the number of subjects utilized in protocols. This decision is typically straightforward, based on assessments of the power of the experiment. Power is related to the ability of the experimental design to distinguish differences of specific magnitudes between treatment groups and is intimately related to measures of variability. Procedures that minimize *random,* within-group/within-subject variability (typically considered error) increase power. In order to achieve a given level of power, fewer subjects are required when there is less variability between subjects (Reduction). Individual animals may respond differently to stressful stimuli, magnifying variation between animals. While a number of different experimental designs, including the use of control groups or within-subjects designs (with appropriate counterbalancing; see Capitanio et al. 2006), can be used to control for individual differences, refinements that yield reductions in overall stress levels across experimental groups of subjects may increase the ability of an experiment to diminish non-hypothesis-related variability across groups.

Suitability

All parties involved in the research endeavor are typically interested in the history (rearing, experimental, and behavioral experience) of the actual animals to be used, especially as it applies to the question "Are these particular animals appropriate models for the hypothesis to be tested?" Economic or financial reasons are typically not sufficient to justify the re-use of animals in multiple protocols. However, for an endangered species like the chimpanzee, in which euthanasia as a management technique is not an option (NRC 1997), expansion of the criteria that might qualify an animal to serve as a subject may be permissible. The animal resources group and in some cases the investigator are typically charged with the responsibility of determining the suitability of individual subjects. The data contained in this issue of *ILAR Journal* suggest that investigators may profit from becoming more involved in selecting individual subjects.

Acclimation

In general, SOPs for acclimation periods and procedures are developed by the animal care group based on the existing empirical literature and their experience with the species and with the procedures. Clearly, the IACUC must understand the protocol and the manipulations to which the ani-

mals are likely to require acclimation. It should also be noted that there is a need for additional investigation concerning these issues, and especially for some of the new "omic" technologies (e.g., transcsriptomic, proteomic, and metabonomic approaches) in which multiple endpoints are evaluated. For example, recent studies have demonstrated significant nuclear magnetic resonance spectroscopic-based changes in urinary metabolite profiles in germ-free rats allowed to acclimate in standard laboratory animal facility conditions (Nicholls et al. 2003).

The length of the period of acclimation necessary for laboratory animals before their use in a research project is one of the most important questions addressed by this issue of *ILAR Journal*. To determine how long animals should be acclimated and to what they need to be acclimated, it is necessary to develop and then evaluate different acclimation periods and procedures. The articles in this issue by Conour and colleagues (2006), Capitanio and colleagues (2006), Meunier (2006), and Smith and Swindle (2006) all provide recommendations for acclimation that are empirically based; data exist and have been cited that support the recommendations in each article. Baseline values that are indicative of nonstressed species-typical responses (physiological and/or behavioral) at the start of experiments are desired. Acclimation periods may differ in length based on the degree of "change" that the animals would be expected to experience. For example, Capitanio and colleagues (2006) advocate 3 mo as an acclimation timeframe for Old World monkeys that are subjected to a large transition from group-housed stock animal to singly-caged experimental subject. Smaller transitions (from a single cage in the stock room to a single cage in an experimental room) should require considerably less acclimation. In contrast, swine "easily become accustomed to daily routines" (Smith and Swindle 2006, p. 361) and typically require only a 1-wk acclimation period.

The acclimation period can and should be used for multiple purposes, not only for health-related quarantine and monitoring but also for behavioral conditioning. This period may include habituation to, desensitization to, and training for procedures that will be involved in experimental use. The correlation is not 1.0, but in general, the longer an animal is likely to serve as a subject, the more effort can—and again should—be devoted to these and other types of conditioning (Bloomsmith et al. 2006).

As mentioned above (see Table 1), certain types of research may require the selection of specific types of subjects to properly test the experimental hypotheses. In these instances, it is prudent to focus conditioning procedures only on those classes of subjects that will be participating in the research project. Additionally, conditioning normally should be aimed specifically at the manipulations these subjects will encounter during experiments. One contradiction to this strategy would be an attempt to establish a pool of feasible subjects for specific types of studies. In this scenario, all animals receive basic acclimation, but certain animals receive additional "training" so that they are available for use as soon as a protocol is approved. Investigators then choose potential subjects only from the pool of "trained" animals, rather than from the colony as a whole. This process is most successful when one employs considerable forethought, planning, and communication to accurately anticipate the needs of the investigator and the requirements of the IACUC. The development of a pool of chimpanzees that will provide unanesthetized blood samples for pharmacokinetic and other studies is one potential example (Schapiro et al. unpublished data).

For many species that live in social groups under natural conditions, social housing in captivity can help buffer some of the effects associated with pre-experimental and experimental manipulations, and may even enhance the research value of the animal model (Capitanio et al. 2006; Smith and Swindle 2006; Tardif et al. 2006). Thus, if social housing can diminish acclimation time for nonhuman primate subjects from 3 mo to 6 wk, it is conceivable that the resulting savings in time and money could "pay for" the potential costs associated with continued social housing.

Homeostasis and Equilibrium

One potential way to view implementation of additional procedures to promote acclimation of experimental subjects is to consider the procedures as attempts to facilitate and enhance the animals' abilities to maintain physiological and behavioral homeostasis. In general, well-acclimated animals are able to deal appropriately with the challenges of the experimental environment. This ability is typically manifested in a transient divergence from equilibrium in response to a manipulation, followed by a gradual return to homeostatic balance. One example may be "fear responses" (behavioral and/or physiological) to cage change-out during the actual change-out, followed by an appropriately timed return to normal/baseline responses. As Capitanio and colleagues (2006) point out, studies in their laboratory (Ruys et al. 2004) have demonstrated that animals appearing to be behaviorally acclimated to a procedure (chairing) are not necessarily physiologically acclimated to that procedure.

Habituation and Desensitization

Although several examples of behavioral factors that can be managed in the research environment have already been presented in this article and the foregoing articles in this issue, both habituation and desensitization deserve additional emphasis. Differing primarily in whether contingent reinforcement is or is not involved, these two procedures are absolutely critical to, and perhaps even define, the successful acclimation of experimental subjects to research procedures and environments. Habituation is passive (no contingent reinforcement is involved), and desensitization is active (animals are reinforced specifically for "interaction" with the target item); however, both techniques are used to change an unpredictable and likely negative experience into a more predictable and potentially positive experience. Use

of the technique will of course expedite acclimation, allowing animals to become accustomed to these situations. For many species, efforts to purposefully and systematically habituate or desensitize subjects to relevant environments and procedures during the pre-experimental acclimation period may result in more valuable experimental data. IACUCs often focus their interest in this activity on areas where special housing and husbandry are used in experimental protocols.

Additional Training of Animals and Time Investment

In addition to habituating and desensitizing animals in the laboratory, it is also possible, and productive, to actively train them to participate in research endeavors. Pigs, dogs, and nonhuman primates are only a few of the species groups that respond well to positive reinforcement training techniques in the laboratory. As mentioned above, training can take place during the pre-experimental acclimation period and is most likely to yield benefits when applied to animals used in "chronic" protocols. In addition to facilitating the collection of data that could be obtained by other, perhaps more stressful means, training also allows for the collection of data that would be impossible by any other means. The collection of routine blood samples from unanesthetized adult chimpanzees is one example.

Investigator Attitudes Toward Increased Preparation

The possibility that some investigators will be resistant to significant changes in preparation procedures must be acknowledged. Investigators typically desire data sets that are comparable over extended time periods. If new preparation procedures produce data that significantly differ from historical norms, even if the new data are "better," longitudinal comparisons of long-term data sets may be affected. This result could necessitate the involvement of the IACUC in the evaluation of proper preparation procedures. As one example of such a dilemma, some of the physiological parameters assayed from our unanesthetized chimpanzees that voluntarily provide blood samples (Lambeth et al. 2005) differ significantly from the published norms for chimpanzees (Ihrig et al. 2001). Virtually all of the published norms are based on blood samples collected from anesthetized chimpanzees. It is not clear whether it would be appropriate for the IACUC to advise investigators to attempt to use only voluntary blood samples.

Investigators trained in the scientific process respond positively to the collection and provision of data. For this reason, IACUCs should develop a "data-based culture." Committees should exhibit flexibility when there is uncertainty concerning the merits of a particular preparation process for experimental protocols. Sometimes what seems logical, especially from an anthropomorphic point of view, does not lead to benefits in animal welfare and can adversely affect data. For example, restraining rodents in nose-only holders is a commonly used method for exposing animals to airborne materials in toxicology experiments. This method has certain deleterious systemic effects on animals, thought to be related in part to stress (King-Herbert et al. 1997). Many inhalation toxicology laboratories had preparative protocols in place to gradually acclimate animals using progressively increased durations of restraint. However, recent experiments using telemetric monitoring of rodents in nose-only holders to evaluate these acclimation procedures found that gradual approaches were actually deleterious to the adaptation outcome (Narciso et al. 2003).

Proper Training of People

As one final point, it is important to mention that additional attention to enhanced preparation procedures for laboratory animals will result in the need for additional knowledge and skills for those who work with the animals. This need will in turn require the provision of additional training for those caring for and working with the animals in the research enterprise. As with many of the cost:benefit analyses in laboratory animal care, IACUCs, investigators, and animal resources groups must be able to understand that the increased costs of additional training are likely to be offset and exceeded by the additional benefits of conducting experiments with animal models of enhanced quality.

Conclusions and Recommendations

In summary, one objective in the planning of this issue of *ILAR Journal,* and of this concluding article, was to provide IACUC members with a resource for determining whether and how preparation of animals for research affect their responsibilities. The articles that comprise this issue clearly demonstrate that preparation of animals is important for optimizing animal welfare as well as for minimizing the occurrence of interanimal variation, thereby strengthening the quality of data for in vivo studies. Both issues are important in the work of IACUCs, but they pose many challenges. The articles in this issue were intended to serve as a starting point for consideration and discussion; certainly they are not and could not represent a set of firm guidelines to be used by IACUCs. It should be clear to readers that flexibility and professional judgment are needed to approach these topics. Questions related to subject selection and acclimation of subjects to experimental housing and procedures are addressed throughout this issue. Guidelines for promoting the acclimation of a number of species to experimental settings are provided. Additional, potentially controversial points are also raised, including the effect of modified preparation of subjects on longitudinal data sets.

The roles of the IACUC in the research endeavor are

multifaceted and continuously evolving. As empirical data are produced that have an impact on additional aspects of animal care and use, it is important for these committees to be able to evaluate and implement procedures and strategies that are likely to affect the collection, analysis, and interpretation of experimental data from animal models in a positive way.

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