Journal of the American Association for Laboratory Animal Science
Copyright 2018
by the American Association for Laboratory Animal Science

Vol 57, No 2
March 2018
Pages 1–6

Contribution of the Breadth and Depth of IACUC Membership to Experimental Design as a Factor in Research Reproducibility

Swapna Mohan,[1,*] Robert W Barbee,[2] and Susan B Silk[1]

The IACUC comprises the key component of animal research oversight at any institution or facility and thus has the responsibility to review and approve proposed animal activities. As the primary oversight unit that ensures the quality of animal welfare and therefore contributes to overall research quality, the IACUC can support reproducibility in research by ensuring rigorous experimental design, standardization of care and management, and assessment of the validity of research. An IACUC that is constituted as required by the Animal Welfare Act and the PHS Policy incorporates a wide range of expertise. Here we explore the contributions of the various IACUC members and discuss how each can help to enhance rigor and mitigate issues regarding irreproducibility in biomedical research involving animals.

Abbreviations: PHS, Public Health Service

Only a small percentage—about 11%—of investigational agents progress to becoming successfully licensed products after clinical trials.[13,19] These discoveries involve extensive preclinical in vitro (cell lines, tissue culture) and in vivo (animal models) studies to validate the investigational compound and to move it to the next level—clinical development. The other 89% of investigational agents fail at one of the several steps of preclinical testing. Many are abandoned when their effects are not adequately reproducible, that is, the research cannot be replicated in another setting or system. Although issues with irreproducibility affect all aspects of biomedical research, animal studies are especially plagued by them. The inability of many studies to be translated from an animal model to human systems or from one animal model to another fosters skepticism about research that uses animal models. This mistrust bolsters the perception that animal models are poor predictors of human disorders and that animal studies are major contributors to the extensive waste of resources accompanying irreproducible research.[13,19,20]

Reproducibility—defined as the replication of results through independent experiments—can be enhanced through rigorous and transparent scientific methods. The application of rigor is required in all stages of scientific research—experimental design, methodology, analysis, interpretation, and reporting. The NIH, as the major funding agency of biomedical research in the United States, has put forth the Initiative to Enhance Research Rigor and Reproducibility, with special emphasis on research design and planning.[8,29,27]

As the key component of animal research oversight at any institution or facility, the IACUC has the responsibility to ensure humane treatment, compliance with federal standards, and accountability in the use of animals in research. However, as members of the scientific community as a whole, IACUC members also have a responsibility (along with the institution and scientists) to promote high-quality scientific research involving animals at their research institutions.[36] Recent publications have addressed the role of IACUC in enhancing reproducibility[10,37,40] by addressing factors such as optimization of animal numbers, harm–benefit analysis, and ethical considerations. This area is where the wide range of expertise found among the members of an IACUC proves useful. One of the many functions of IACUC is to review and approve proposed animal activities, which includes the ability to require protocol modifications from researchers. Here we discuss ways in which the various IACUC members can contribute to this review and ensure rigor in the experimental design.

IACUC Membership and Contribution to Research Success

The Public Health Service (PHS) *Policy on Humane Care and Use of Laboratory Animals* (*PHS Policy*) instructs in section IV.A.3.b. (1)-(4) that "The committee shall consist of no fewer than five members, and shall include at least:

(1) one Doctor of Veterinary Medicine, with training or experience in laboratory animal science and medicine, who has direct or delegated program authority and responsibility for activities involving animals at the institution;
(2) one practicing scientist experienced in research involving animals;
(3) one member whose primary concerns are in a nonscientific area (for example, ethicist, lawyer, and member of the clergy); and
(4) "one individual who is not affiliated with the institution in any way other than as a member of the IACUC, and is not a member of the immediate family of a person who is affiliated with the institution."[32]

The *PHS Policy* then goes on to say (in IV.A.3.c.) that "An individual who meets the requirements of more than one of the categories … may fulfill more than one requirement. However, no committee may consist of fewer than five members."[32] This

Received: 12 Sept 2017. Revision requested: 19 Oct 2017. Accepted: 20 Nov 2017.
[1]Office of Laboratory Animal Welfare, National Institutes of Health, Bethesda, Maryland; and [2]Office of Research and Innovation, Virginia Commonwealth University, Richmond, Virginia
[*]Corresponding author. Email: swapna.mohan@nih.gov

requirement ensures that each IACUC is a unit composed of a variety of experts, and therefore, contributes a unique advantage to the institutional culture. An IACUC at an institution can contribute to the management of conflicts of interest, compliance with federal standards, promotion of good research practices, and facilitate training, in addition to its role in ensuring excellent animal welfare.

The *US Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training* (*US Government Principles*)[31] mandates that "Procedures involving animals should be designed and performed with due consideration of their relevance to human or animal health, the advancement of knowledge, or the good of society." The *US Government Principles* also states that "The animals selected for a procedure should be of an appropriate species and quality and the minimum number required to obtain valid results."[31]

Knowledge is advanced by studies that are both rigorously designed and reproducible in various settings. Moreover, the quality and validity of a study depend, in part, on robust experimental design, methodology, and consistency in its planning and execution. These features mean that, in addition to animal welfare, the IACUC has some authority to review the scientific merit and quality of a study. The *US Government Principles* calls for an evaluation of the relevance of a procedure to human or animal health, the advancement of knowledge, or the good of society. In addition, the *PHS Policy* uses language such as "consistent with sound research design," "rationale for involving animals," and "in the conduct of scientifically valuable research" when describing the review of proposals by the IACUC (sections IV.C. I and IV.D. 1.)[26,32,31] Although an IACUC is not required to assess the scientific quality of research projects, it must ensure that animals are not used needlessly in nonproductive research. Thus weighing the scientific quality of a proposal has the benefit of promoting good practices, which can lead to reproducible results, in addition to improved animal welfare.

Limitations of Animal Research

The unpredictability of the translation of animal research to human clinical trials has been attributed to various factors, including publication bias (negative results omitted),[39] poor study design and methodology,[20] environmental variables,[7] and the inability of animal models to adequately replicate human physiology and disease processes.[33] Some of this variability is due to unknown or uncontrolled biologic variables, and other variables might be introduced as a result of suboptimal or detrimental research practices.[23,37] Figure 1 summarizes the most common limitations of animal research that can lead to issues regarding experimental reproducibility.

Although unintentional, partiality in research reporting can be introduced due to the pressure to publish novel and exciting results. Negative or neutral results often may be underreported or ignored in favor of those that support the scientific hypothesis of the study.[1] A 2010 study on stroke research in animal models revealed significant overstatement of the efficacy of several interventions due to publication bias.[34] Although prevalent in almost all areas of preclinical biomedical research, the effect of such bias in animal research is compounded by the lack of standardization in reporting animal studies.[38] In 2010, the UK National Centre for the Replacement, Refinement, and Reduction of Animals in Research (NC3Rs) proposed the ARRIVE guidelines (Animals in Research: Reporting *In Vivo* Experiments) for reporting preclinical animal research. These guidelines include suggestions regarding statistical assessment, the disclosure of unexpected outcomes, and checklists for variables that should be addressed in scientific reports.[18] The scientific community is beginning to embrace these and other guidelines to standardize research reporting and to eliminate bias.

In addition, lack of standardization affects study design, methodology, and analysis. For example, the ALS Therapy Development Institute reported that rigorous retesting of many of the published potential treatments for amyotrophic lateral sclerosis failed to replicate the published results.[34] Such discrepancies were attributed to sex-associated bias, clustering within litters, and censoring criteria that significantly increased 'noise' or confounding variables within the results.[34,38] The recommendations by ALS TDI for improving preclinical study design were to redefine the criteria for analyzing study results, identifying and discarding outliers, normalizing for the sex of animal subjects, assigning animals to study groups in a way to prevent clustering of siblings, and to track genes that may not be inherited reliably. These and other studies demonstrate the importance of careful planning, characterization, and establishment of standards and guidelines for study design and reporting.

The standardization of resources and methodology are important factors to consider when dealing with issues regarding reproducibility. This standardization involves using established biologic and chemical resources such as reagents, cell lines, and antibodies. Treatment regimens are another, often overlooked, part of standardization of resources and methodology. Both the persistence of pain and the various analgesics used to treat pain can affect research outcomes significantly.[5] Publication guidelines that focus on potential sources of bias should also address the potential for pain and pain relief to introduce reproducibility issues in the studies.

However, even with careful planning and execution of experiments, variability in environmental factors can affect the reproducibility of animal research. For example, some studies report variation in results when environmental temperatures are altered.[14] Even a mild reduction in temperatures can significantly bias conclusions[14] drawn from studies in murine models of human diseases, such as obesity, cardiovascular disorders, inflammation, and cancer.

Nutrition is another major factor that contributes to variability in animal research. Investigators assume that standard animal feeds from commercial sources are uniform in their content. However, individual components of an animal's diet can affect experimental endpoints. For example, variation in flavonoid content and quality between batches of commercial mouse chow can introduce variables in cancer studies.[17] In addition, increasing evidence indicates that the gut microbiome plays a vital role in phenotype development in commonly used laboratory mice.[9] The gut microbiome in these animals can vary depending on the vendor or batch of mouse chow fed to them.

However, even when all environmental deviations are minimized so that animal models are exposed to optimal environmental conditions, differences in basic physiology remain to be considered. Although animal models provide analogous systems to understand disease progression, drug effects, and molecular mechanisms, it is important to consider the extent to which physiologic information is transferable between animal models and translatable to humans. Incomplete or inaccurate validation of animal models can reduce this transferability. For example, a species with apparently high validity (similarity in biology and disease symptoms) might not provide an accurate prediction of clinical effects, due to differences in disease mechanisms.

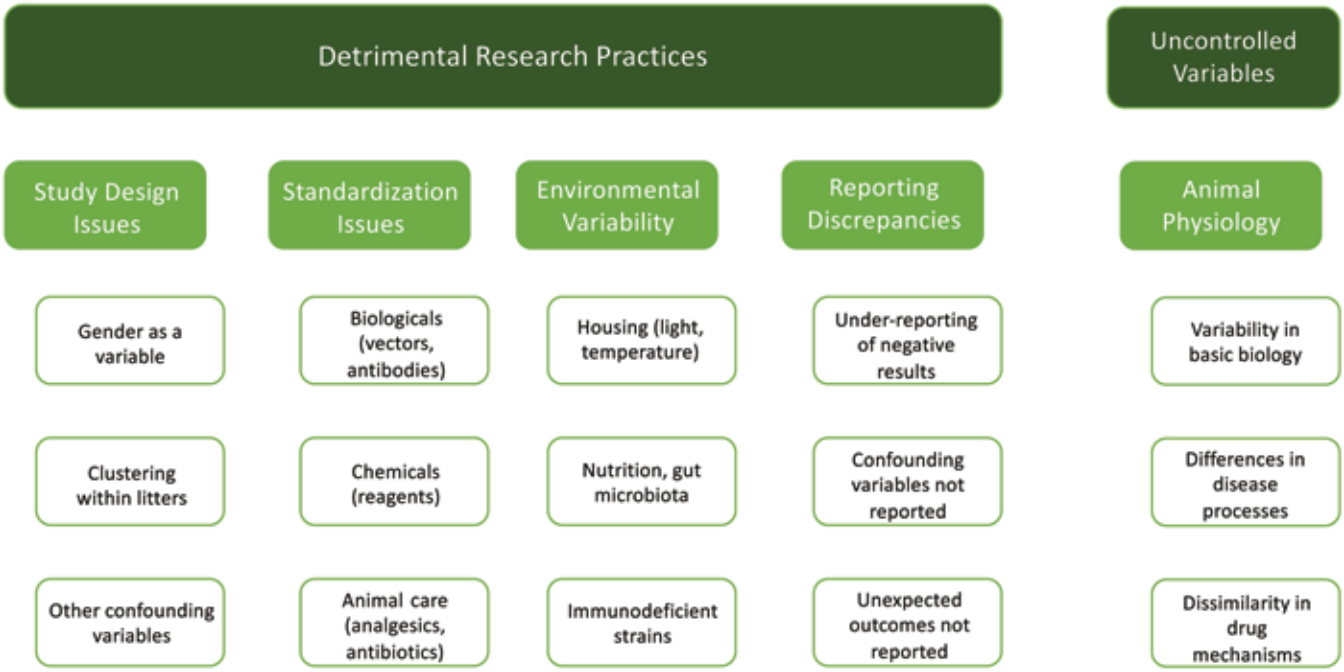

Figure 1. Common limitations of animal research that can lead to poor experimental reproducibility.

How Can the IACUC Help?

Poorly controlled or unknown variability can degrade the standard and quality of scientific research. In addition to ensuring high-quality animal care, the IACUC can ensure high standard of animal research at their institutions by providing appropriate guidance for design, documentation, and consistency in animal research.[40]

In June 2015, NIH issued a Guide Notice addressing the use of rigor and transparency to increase reproducibility in research.[27] To enhance reproducibility in scientific research, 4 major areas were identified, and applicants are asked to appropriately address each area in their grant proposals. These areas are: 1) the scientific premise of the proposed research; 2) rigorous experimental design for robust and unbiased results; 3) consideration of relevant biologic variables; and 4) authentication of key biologic and chemical resources. Consideration of these key areas is designed to help with the assessment and replication of results, both before and after publication. To address these areas, the field of preclinical research is adopting several practices, such as standardization of research practices (for example, using standard operating procedures or publicly accessible protocols for experiments), and establishing reporting guidelines (for example, the inclusion of pain and stress relief and management in the Methods section of publications). However, the burden of considering and addressing these areas of research should not fall on the scientists alone. The IACUC can contribute greatly to the planning and implementation of research involving animals as they help maintain the rigor and transparency standards of the institution.

Although the primary focus of protocol reviews by the IACUC has been humane use of animals, application of the 3Rs, and minimization of pain and distress in animals, IACUC also have the authority to evaluate scientific elements of the protocol that pertain to the use of animals in research. The *Guide for the Care and Use of Laboratory Animals* (the *Guide*) offers the following guidance on scientific merit review by the IACUC: "… the committee members should evaluate scientific elements of the protocol as they relate to the welfare and use of the animals. For example, hypothesis testing, sample size, group numbers, and adequacy of controls can relate directly to the prevention of unnecessary animal use or duplication of experiments."[15] The *Guide* goes on to say that "in the absence of evidence of a formal scientific merit review, the IACUC may consider conducting or requesting such a review" and that the IACUC may seek "… input from outside experts …" when necessary.[15,35]

These provisions do not mean that the IACUC is expected to perform scientific reviews of the same depth as those performed by peer-review committees for grant proposals. Nor do they mean that the protocol review process should be made more burdensome to the IACUC by adding more tasks. Instead, better delineation and understanding of the roles of IACUC members would ensure that members are fulfilling their individual roles in the most efficient way. The IACUC chair can ensure that concerns of all members—including those members who are not well versed in the scientific process—are heard and adequately addressed.[35] Similarly, the Institutional Official (IO) may provide guidance on the bigger goals of the institution in maintaining rigor and reproducibility in its scientific research. Figure 2 explores some of the possible contributions of each IACUC member category toward reproducibility in research.

Role of the Veterinarian

The veterinarian has a vital role in the planning and implementation of research studies. When included early in the planning stage, as required in the Animal Welfare Act Regulations for pain category D or E procedures[2] and the *Guide*,[15] the veterinarian can provide advice on choice of animal models, any physiologic parameters that might influence the study, and the establishment of behavioral indicators. In addition, the veterinarian can provide guidance on husbandry practices and other factors (for example, treatment regimens) that might affect experiments so that the researcher can plan accordingly.

For example, veterinarians are expected to help assess and monitor the pain management strategy for animal studies. Although pain relief might primarily be seen as an aspect of animal welfare, studies show the important roles pain and pain

IACUC member	Member's contribution to experimental rigor and reproducibility
Veterinarian	Compares animal models Assesses physiologic parameters Establishes humane and scientifically appropriate endpoints Gives guidance on more 'natural' conditions, enrichment Provides guidance on husbandry practices that might influence research Makes treatment regimens as uniform as possible Provides skill assessment and training to investigators
Scientist	Gives guidance on research design, sample size, use of controls Offers instruction regarding science-oriented husbandry Provides input regarding the availability of less invasive procedures, interventions, and other refinement measures
Nonaffiliated or nonscientist	Ensures transparency Ensures complete and understandable description of animal activities Ensures that alternatives have been given adequate consideration
Others	Assist with statistical design Comment regarding validity of animals models Ensure uniformity across experiments Provides other guidance

Figure 2. Potential roles of IACUC members regarding enhancing the reproducibility of research during research design. Overall, IACUC provide guidance during the planning and design of animal experiments.

management in experimental reproducibility. Because different analgesics affect research outcomes differently, some scientists assume that removing this variable entirely will help to standardize experiments.[6] However, untreated pain is a stressor that can affect the immune system, behavior, body condition score, and metabolism. The veterinarian might be able to eliminate some of these potential effects on data by standardizing care and treatment regimens, introducing better methods of handling and restraint to reduce anxiety,[12] and training investigators regarding behavioral indicators (for example, grimace scales) to manage pain in animals.[21,22] Furthermore, these indicators can help with the establishment of humane experimental endpoints. The establishment and application of consistent endpoints are key to successfully replicating a study.[40] While formulating and defining endpoints, the veterinarian and researchers can work together to ensure alleviation of pain and distress and to chart clinical observations that define scientifically appropriate endpoints.[30]

In addition, the veterinarian can provide guidance on species-appropriate enrichment within research facilities. Published studies on animal housing enrichment demonstrate that provision of enrichment not only enhances animal welfare but also positively influences experimental validity and reproducibility.[4] Veterinarians' understanding of biology and behavior (as well as factors such as temperature, feed, and so forth, as discussed earlier) ensures that the enrichment methods are standardized to reduce variability, particularly in studies with behavioral components.[41]

Finally, the veterinarian is in a position to assess investigators' skill sets and to provide appropriate training regarding techniques of surgical manipulation, asepsis, and pain relief. Addressing these aspects helps to maintain consistency in procedures and to maximize reproducibility.[15,40]

Role of the Scientist

A 2015 workshop of the Institute for Laboratory Animal Research offered guidance on combating irreproducibility in animal research. Some of the measures proposed, such as randomization and blinded assessment of outcomes,[24] can be suggested by the IACUC scientists, when appropriate, during evaluation of research protocols.

Scientific members on the IACUC can provide input on experimental variables (for example, sample size determination, the use of controls), husbandry issues that might influence research (for example, noise, vibration, and single compared with social housing), and refinement measures (for example, less invasive procedures and interventions), and whether these measures are likely to affect the scientific validity of the experiment. The *Guide* requires consideration of "the availability and appropriateness of less invasive procedures."[15] This aspect of refinement can have significant effects on study outcomes, given that pain from invasive procedures can affect immune function, metabolism, food intake, behavior, and general physiology.[5]

Because they are most familiar with the resources present at their institutions, scientists on the IACUC can help implement measures such as standardization of key biologic and chemical resources, including cell lines, specialty chemicals, antibodies, pharmaceutical products, and other biologics. From their experience, scientist IACUC members will be able to establish acceptable performance-based criteria and tests for the analysis of experimental observations and results; these criteria and tests, in turn, will help to improve study design.

Role of the Nonscientist or Nonaffiliated Member

The nonaffiliated and nonscientist member(s) serve the necessary function of introducing the community or public perspective on animal welfare and research. The *PHS Policy* (section IV.A.3.b (3, 4)) describes the nonaffiliated member as an "individual who is not affiliated with the institution in any way other than as a member of the IACUC, and is not a member of the immediate family of a person who is affiliated with the institution;" the nonscientific member is an individual "whose primary concerns are in a nonscientific area."[32] The *Animal Care Resource Guide* defines the nonaffiliated member as someone who "provide representation for general community interests in the proper care and treatment of animals."[3] Therefore, the participation of these members as impartial public representatives ensures transparency in all IACUC functions. In addition, nonscientific and nonaffiliated IACUC members can ensure that the descriptions of proposed animal activities

are complete and understandable to everyone, by requesting the use of nonscientific language.[35] In the context of their role of providing community input, nonscientific and nonaffiliated IACUC members can encourage the debate toward reproducibility and better experimental rigor.

Statistical Assistance

The *Guide* states that for protocol review by the IACUC "… the number of animals and experimental group sizes should be statistically justified (e.g., provision of a power analysis)." The purpose of a statistical justification during IACUC reviews is to reduce the number of animals to the fewest needed to obtain statistically significant data, to refine animal care and use to minimize pain and distress, and to enhance animal wellbeing.[15] However, the IACUC often is expected to find a balance when making this decision: requirement of repetitions of a study to prove its validity and reproducibility and, simultaneously, prevention of unnecessary animal use or duplication of experiments.

Although the IACUC is not required to provide statistical consultation for ensuring research rigor, it is nevertheless helpful to have members with statistical experience on the committee; these members then might be able to assist with the rigor and reproducibility strategy that increasing numbers of journals seek in submitted manuscripts. Multiple publications suggest that improved statistical design is needed to optimize the design, conduct, and analysis of studies.[11,16]

Role of the Institutional Official

The preamble to the *US Government Principles* states: "Whenever US Government agencies … perform or sponsor such procedures [involving the use of vertebrate animals]; the responsible Institutional Official shall ensure that these principles are adhered to."[31]

The Institutional Official has the responsibility to ensure a high standard of animal care and use at his or her institution and to promote a culture of good scientific practices and compliance. The Institutional Official can set the standard for larger goals of institutional rigor and support the IACUC in addressing reproducibility during study design and planning. And finally, the Institutional Official can support the establishment of a high-functioning IACUC at his or her institution. The *PHS Policy* states that "the Chief Executive Officer [of the research institution] shall appoint an … IACUC." (*PHS Policy* IV.A.3 (a)) By appointing appropriate members to the IACUC, the Institutional Official can ensure that the scientific breadth and depth within the IACUC is congruent with the types of live-animal procedures performed at the institution. Having a diverse IACUC becomes increasingly relevant as the global research environment becomes more complex.[23]

Conclusions

Valid, reproducible data from animal research are inseparably linked to standardization of care and use of animals and high animal welfare. Animals experiencing stress, boredom, and anxiety may yield unreliable data. The ultimate goal of preclinical animal research is to apply our understanding of biologic systems to other species, particularly humans. When the results obtained from animal research are not translatable to humans, then ethically justifying the use of animals in research becomes increasingly difficult. The IACUC's primary responsibility is to ensure humane care and use of research animals. The IACUC is in a unique position to assess proposed research and evaluate the merit of each study as it pertains to the 3Rs. Keeping scientific rigor and reproducibility in mind when evaluating studies would hold the institution's research to higher standards, in addition to ensuring a high level of animal welfare.

As part of its ongoing efforts regarding scientific rigor and reproducibility, NIH has a helpful resource for IACUCs regarding the evaluation of rigor and reproducibility both in new grant applications (for IACUC approval) and ongoing protocols.[28] Relevant topics include the consideration of biologic variables such as sex and age and authentication of key resources. Finally, some institutes are now funding training efforts that address scientific reproducibility and rigor.[25]

Biomedical research uses animal models to investigate highly complex biologic systems. However, this complexity introduces variables. Variability that is poorly understood, combined with suboptimal research practices such as flawed experimental planning, inadequate controls, and insufficient standardization of resources, can contribute to irreproducibility. In turn, these deficiencies can make animals poor predictors of human physiologic processes. The issue of experimental irreproducibility concerns the entire scientific community—researchers, institutions, federal agencies, professional associations, and publishers. As part of the trio (that is, oversight board, institution, and researcher) that ensures the quality of animal research at an institution, IACUC can support reproducibility in research by educating researchers—and IACUC members themselves—regarding rigorous experimental design, standardization of resources, and assessment of the validity of research data.

References

1. **Alberts B, Kirschner MW, Tilghman S, Varmus H.** 2014. Rescuing US biomedical research from its systemic flaws. Proc Natl Acad Sci USA **111:**5773–5777.
2. **Animal Welfare Act and Regulations.** 2017. 7 CFR § 2143 (a)(3)(C)(i).
3. **Animal Welfare Act and Regulations.** 2017. 9 CFR § 2.31 (b)(3)(ii).
4. **Bayne K, Wurbel H.** 2014. The impact of environmental enrichment on the outcome variability and scientific validity of laboratory animal studies. Rev Sci Tech **33:**273–280.
5. **Carbone L, Austin J.** 2016. Pain and laboratory animals: publication practices for better data reproducibility and better animal welfare. PLoS One **11:**e0155001.
6. **Chalmers I, Glasziou P.** 2009. Avoidable waste in the production and reporting of research evidence. Lancet **374:**86–89.
7. **Chesler EJ, Wilson SG, Lariviere WR, Rodriguez-Zas SL, Mogil JS.** 2002. Identification and ranking of genetic and laboratory environment factors influencing a behavioral trait, thermal nociception, via computational analysis of a large data archive. Neurosci Biobehav Rev **26:**907–923.
8. **Collins FS, Tabak LA.** 2014. Policy: NIH plans to enhance reproducibility. Nature **505:**612–613.
9. **Ericsson AC, Davis JW, Spollen W, Bivens N, Givan S, Hagan CE, McIntosh M, Franklin CL.** 2015. Effects of vendor and genetic background on the composition of the fecal microbiota of inbred mice. PLoS One **10:**e0116704.
10. **Everitt JI, Berridge BR.** 2017. The role of the IACUC in the design and conduct of animal experiments that contribute to translational success. ILAR J **58:**129–134.
11. **Glasziou P, Macleod M, Chalmers I, Ioannidis JP, Al-Shahi Salman R, Chan AW.** 2014. Research: increasing value, reducing waste - Authors' reply. Lancet **383:**1126–1127.
12. **Graham ML, Prescott MJ.** 2015. The multifactorial role of the 3Rs in shifting the harm-benefit analysis in animal models of disease. Eur J Pharmacol **759:**19–29.
13. **Henderson VC, Kimmelman J, Fergusson D, Grimshaw JM, Hackam DG.** 2013. Threats to validity in the design and conduct of preclinical efficacy studies: a systematic review of guidelines for in vivo animal experiments. PLoS Med **10:**1–14.

14. **Hylander BL, Repasky EA.** 2016. Thermoneutrality, mice, and cancer: a heated opinion. Trends Cancer **2:**166–175.
15. **Institute for Laboratory Animal Research.** 2011. Guide for the care and use of laboratory animals, 8th ed.Washington (DC): National Academies Press.
16. **Ioannidis JP, Greenland S, Hlatky MA, Khoury MJ, Macleod MR, Moher D, Schulz KF, Tibshirani R.** 2014. Increasing value and reducing waste in research design, conduct, and analysis. Lancet **383:**166–175.
17. **Jensen MN, Ritskes-Hoitinga M.** 2007. How isoflavone levels in common rodent diets can interfere with the value of animal models and with experimental results. Lab Anim **41:**1–18.
18. **Kilkenny C, Browne WJ, Cuthill IC, Emerson M, Altman DG.** 2010. Improving bioscience research reporting: The ARRIVE Guidelines for Reporting Animal Research. PLoS Biol **8:**1–5.
19. **Kola I, Landis J.** 2004. Can the pharmaceutical industry reduce attrition rates? Nat Rev Drug Discov **3:**711–715.
20. **Landis SC, Amara SG, Asadullah K, Austin CP, Blumenstein R, Bradley EW, Crystal RG, Darnell RB, Ferrante RJ, Fillit H, Finkelstein R, Fisher M, Gendelman HE, Golub RM, Goudreau JL, Gross RA, Gubitz AK, Hesterlee SE, Howells DW, Huguenard J, Kelner K, Koroshetz W, Krainc D, Lazic SE, Levine MS, Macleod MR, McCall JM, Moxley RT 3rd, Narasimhan K, Noble LJ, Perrin S, Porter JD, Steward O, Unger E, Utz U, Silberberg SD.** 2012. A call for transparent reporting to optimize the predictive value of preclinical research. Nature **490:**187–191.
21. **Leach M**. [Internet]. 2013. Using facial expressions of pain in animals. NC3R 2013 research review. [Cited 27 April 2017]. Available at: https://www.nc3rs.org.uk/using-facial-expressions-pain-animals
22. **Leach MC, Klaus K, Miller AL, Scotto di Perrotolo M, Sotocinal SG, Flecknell PA.** 2012. The assessment of postvasectomy pain in mice using behaviour and the mouse grimace scale. PLoS One **7:**e35656.
23. **National Academies of Sciences Engineering and Medicine.** 2017. Fostering integrity in research. Washington (DC): National Academies Press.
24. **National Academies Press**. [Internet]. 2015. Reproducibility issues in research with animals and animal models: workshop in brief. [Cited 27 March 2017] Available at: https://www.nap.edu/catalog/21835/reproducibility-issues-in-research-with-animals-and-animal-models-workshop.
25. **National Institute of General Medical Sciences.** [Internet]. 2018. Clearinghouse for training modules to enhance data reproducibility. [Cited 11 September 2017]. Available at: https://www.nigms.nih.gov/training/pages/clearinghouse-for-training-modules-to-enhance-data-reproducibility.aspx.
26. **National Institutes of Health.** [Internet]. 1991. The public health service responds to commonly asked questions. [Cited 6 August 2017]. Available at: https://grants.nih.gov/grants/olaw/references/ilar91.htm
27. **National Intitutes of Health.** [Internet]. 2015. Enhancing reproducibility through rigor and transparency. notice number: NOT-OD-15-103. [Cited 6 April 2017]. Available at: https://grants.nih.gov/grants/guide/notice-files/NOT-OD-15-103.html.
28. **National Institutes of Health.** [Internet]. 2016. FAQs on Rigor and Transparency. [Cited 25 October 2017]. Available at: https://grants.nih.gov/reproducibility/faqs.htm
29. **National Institutes of Health.** [Internet]. 2018. Rigor and reproducibility. [Cited 25 October 2017]. Available at: https://www.nih.gov/research-training/rigor-reproducibility.
30. **National Research Council (US) Committee on Recognition and Alleviation of Pain in Laboratory Animals.** [Internet]. 2009. Recognition and alleviation of pain in laboratory animals.Washington (DC): National Academies Press. Humane Endpoints for animals in pain. [Cited 30 October 2017]. Available at: https://www.ncbi.nlm.nih.gov/books/NBK32660/
31. **Office of Laboratory Animal Welfare**. [Internet]. 2013. U.S. Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training. Federal Register. [Cited 8 September 2017]. Available at: https://grants.nih.gov/grants/olaw/tutorial/relevant.htm
32. **Office of Laboratory Animal Welfare**. [Internet] 2015. Public Health Service policy on humane care and use of laboratory animals. [Cited 12 September 2017]. Available at: https://grants.nih.gov/grants/olaw/olaw.htm.
33. **Perel P, Roberts I, Sena E, Wheble P, Briscoe C, Sandercock P, Macleod M, Mignini LE, Jayaram P, Khan KS.** 2007. Comparison of treatment effects between animal experiments and clinical trials: systematic review. BMJ **334:**1–6.
34. **Perrin S.** 2014. Preclinical research: make mouse studies work. Nature **507:**423–425.
35. **Petrie WK, Wallace SL, editors.** 2015. The care and feeding of an IACUC: the organization and management of an institutional animal care and use committee, 2nd ed. https://doi.org/10.1201/b18269
36. **Prentice ED, Crouse DA, Mann MD.** 1992. Scientific merit review: the role of the IACUC. Ilar News **34:**15–19.
37. **Pritt SL, Hammer RE.** 2017. The interplay of ethics, animal welfare, and IACUC oversight on the reproducibility of animal studies. Comp Med **67:**101–105.
38. **Scott S, Kranz JE, Cole J, Lincecum JM, Thompson K, Kelly N, Bostrom A, Theodoss J, Al-Nakhala BM, Vieira FG, Ramasubbu J, Heywood JA.** 2008. Design, power, and interpretation of studies in the standard murine model of ALS. Amyotroph Lateral Scler **9:**4–15.
39. **Sena ES, van der Worp HB, Bath PM, Howells DW, Macleod MR.** 2010. Publication bias in reports of animal stroke studies leads to major overstatement of efficacy. PLoS Biol **8:**1–8.
40. **Silverman J, Macy J, Preisig PA.** 2017. The role of the IACUC in ensuring research reproducibility. Lab Anim (NY) **46:**129–135.
41. **Toth LA, Kregel K, Leon L, Musch TI.** 2011. Environmental enrichment of laboratory rodents: the answer depends on the question. Comp Med **61:**314–321.