Rigor & Reproducibility: Back to Basics

October 17, 2015

NIH Regional Seminar

Judy Hewitt, Ph.D.

On Detail to OER

NIH National Institutes of Health
Office of Extramural Research

Learning Objectives

- Describe the issue of reproducibility and NIH plans to address it
- Summarize changes to application instructions and review criteria for NIH grants
- Explain how the policies behind rigor and transparency will impact different types of grants along with the implementation timeline

The Reproducibility Challenge

- Noted by research community; in multiple publications
  - Across research areas
  - Especially in preclinical research

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  - Across research areas
  - Especially research

Beware the creeping cracks of bias

Evidence is mounting that research is riddled with systematic errors. Left unchecked, this could erode public trust, warns **Daniel Sarewitz.**

Why animal research needs to improve

Many of the studies that use animals to model human diseases are too small and too prone to bias to be trusted, says **Malcolm Macleod.**

Science & technology Culture

Unreliable research

Trouble at the lab

Scientists like to think of science as self-correcting. To an alarming degree, it is not

Oct 19th 2013 | From the print edition

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Believe it or not: how much can we rely on published data on potential drug targets?

Florian Prinz, Thomas Schlange and Khusru Asadullah

False-Positive Psychology: Undisclosed Flexibility in Data Collection and Analysis Allows Presenting Anything as Significant

Raise standards for preclinical cancer research

C. Glenn Begley and Lee M. Ellis propose how methods, publications and incentives must change if patients are to benefit.

Drug targets slip-sliding away

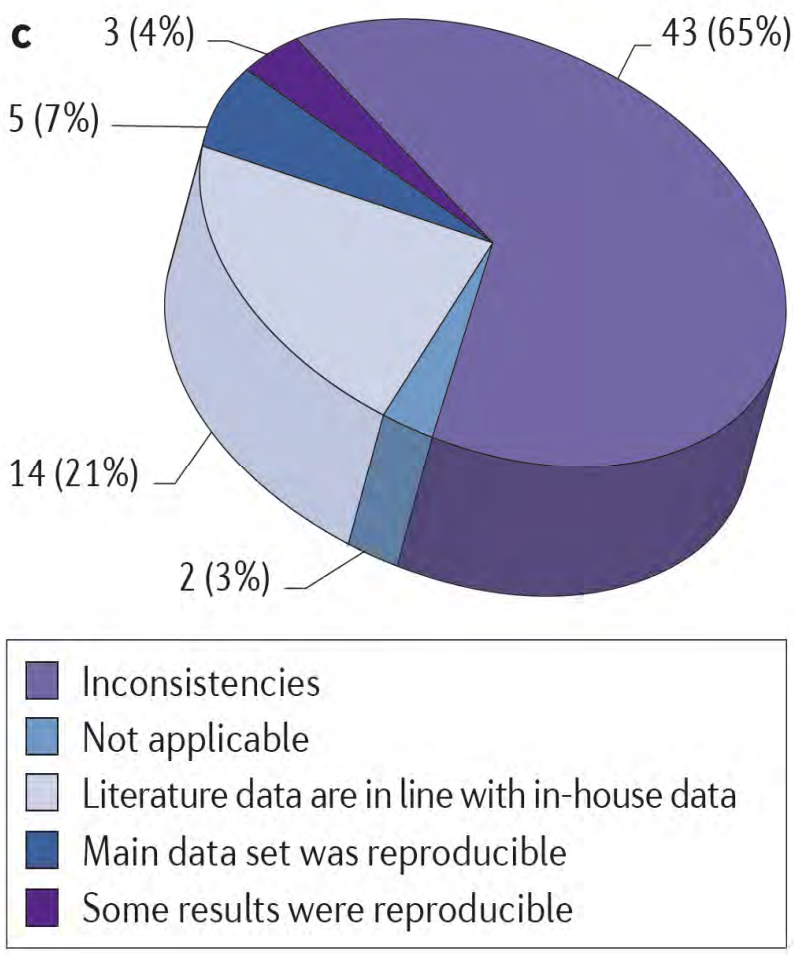
The starting point for many drug discovery programs is a published report on a new drug target. Assessing the reliability of such papers requires a nuanced view of the process of scientific discovery and publication.

Reforming Science: Methodological and Cultural Reforms

Believe it or not: how much can we rely on published data on potential drug targets?

Prinz, Schlange and Asadullah
Bayer HealthCare

Nature Reviews Drug Discovery
2011; 10:712-713



PERSPECTIVE

doi:10.1038/nature11556

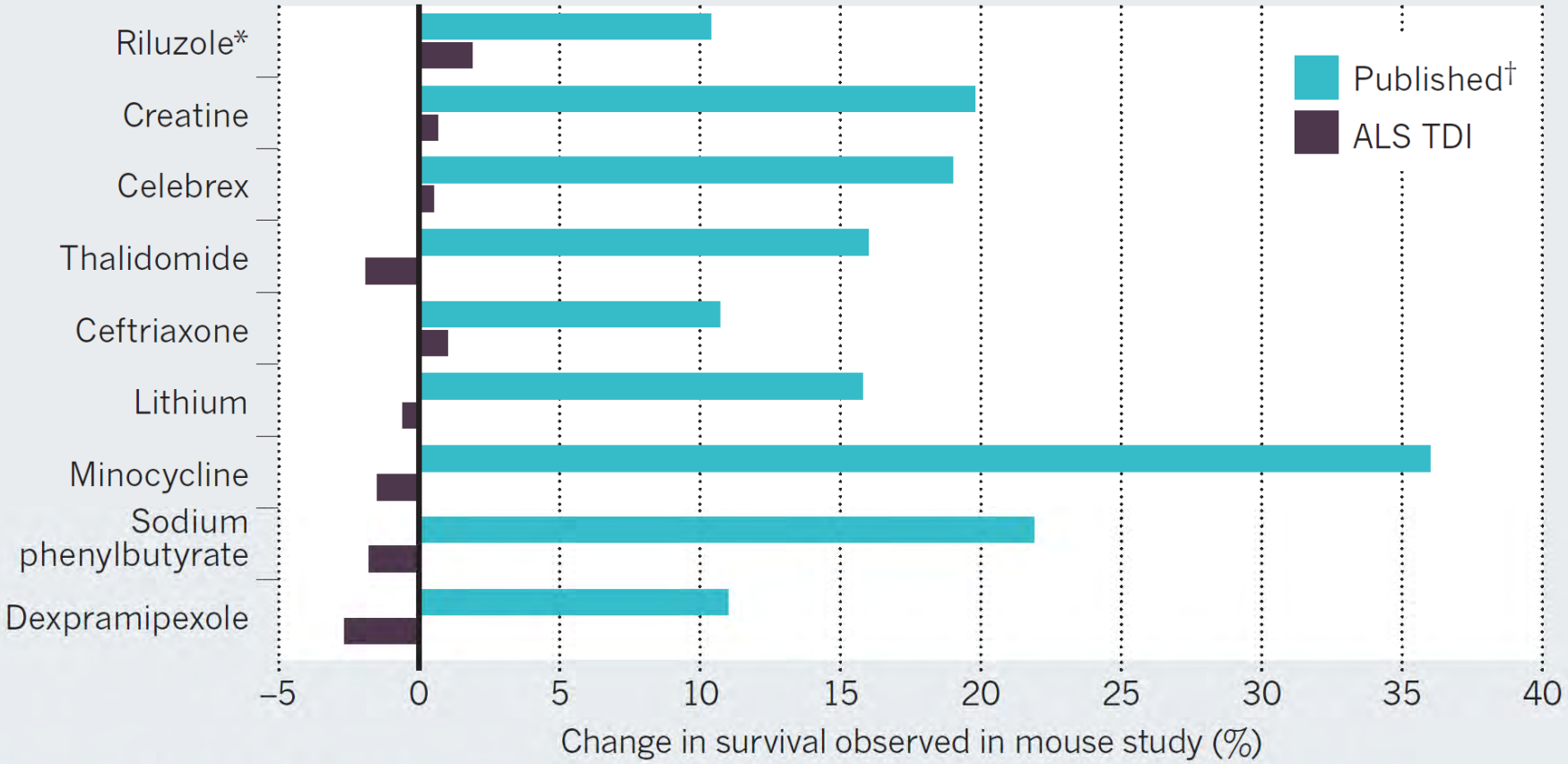
A call for transparent reporting to optimize the predictive value of preclinical research

Story C. Landis[1], Susan G. Amara[2], Khusru Asadullah[3], Chris P. Austin[4], Robi Blumenstein[5], Eileen W. Bradley[6], Ronald G. Crystal[7], Robert B. Darnell[8], Robert J. Ferrante[9], Howard Fillit[10], Robert Finkelstein[1], Marc Fisher[11], Howard E. Gendelman[12], Robert M. Golub[13], John L. Goudreau[14], Robert A. Gross[15], Amelie K. Gubitz[1], Sharon E. Hesterlee[16], David W. Howells[17], John Huguenard[18], Katrina Kelner[19], Walter Koroshetz[1], Dimitri Krainc[20], Stanley E. Lazic[21], Michael S. Levine[22], Malcolm R. Macleod[23], John M. McCall[24], Richard T. Moxley III[25], Kalyani Narasimhan[26], Linda J. Noble[27], Steve Perrin[28], John D. Porter[1], Oswald Steward[29], Ellis Unger[30], Ursula Utz[1] & Shai D. Silberberg[1]

The US National Institute of Neurological Disorders and Stroke convened major stakeholders in June 2012 to discuss how to improve the methodological reporting of animal studies in grant applications and publications. The main workshop recommendation is that at a minimum studies should report on sample-size estimation, whether and how animals were randomized, whether investigators were blind to the treatment, and the handling of data. We recognize that achieving a meaningful improvement in the quality of reporting will require a concerted effort by investigators, reviewers, funding agencies and journal editors. Requiring better reporting of animal studies will raise awareness of the importance of rigorous study design to accelerate scientific progress.

DUE DILIGENCE, OVERDUE

Results of rigorous animal tests by the Amyotrophic Lateral Sclerosis Therapy Development Institute (ALS TDI) are less promising than those published. All these compounds have disappointed in human testing.



*Although riluzole is the only drug currently approved by the US Food and Drug Administration for ALS, our work showed no survival benefit.
†References for published studies can be found in supplementary information at go.nature.com/hf4jf6.

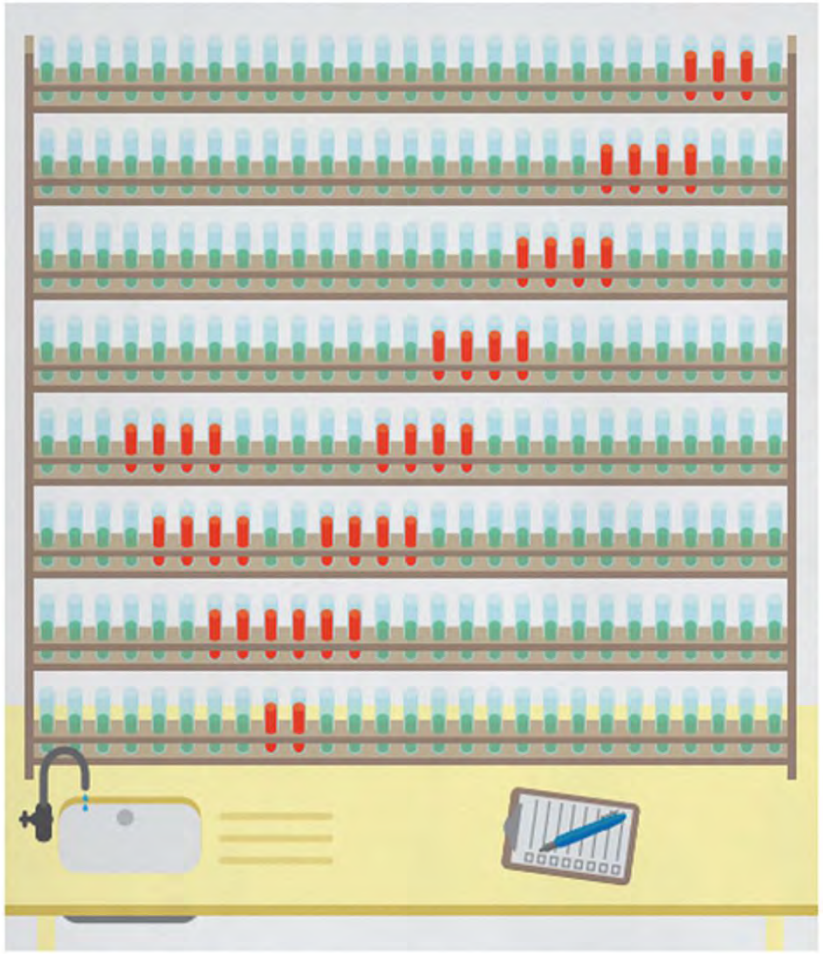
Perrin, *Nature* 2014; 507: 423-425

Good Experimental Design (and Reporting) Underlies Rigor and Reproducibility of Findings

Five requirements for a "good" experimental design:

- Be unbiased
- Have high precision
- Have a wide range of applicability
- Be simple
- Have the ability to calculate uncertainty

COX, D.R. *Planning Experiments*, John Wiley and Sons, New York, 1958.



CHRIS RYAN/NATURE

NIH plans to enhance reproducibility

Francis S. Collins and **Lawrence A. Tabak** discuss initiatives that the US National Institutes of Health is exploring to restore the self-correcting nature of preclinical research.

A growing chorus of concern, from scientists and laypeople, contends that the complex system for ensuring

shorter term, however, the checks and balances that once ensured scientific fidelity have been hobbled. This has compromised

outnumbered by the hundreds of thousands published each year in good faith.

Instead, a complex array of other factors seems to have contributed to the lack of reproducibility. Factors include poor training of researchers in experimental design; increased emphasis on making provocative statements rather than presenting technical details; and publications that do not report basic elements of experimental design[4]. Crucial experimental design elements that are all too frequently ignored include blinding, randomization, replication, sample-size calculation and the effect of sex differences.

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tenure, and in e
rewards[6].

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not published.
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papers that poin
viously published work. Further compounding the problem is the difficulty of accessing unpublished data — and the failure of funding agencies to establish or enforce policies that insist on data access.

> *"Efforts by the NIH alone will not be sufficient to effect real change in this unhealthy environment."*

PRECLINICAL PROBLEMS

Reproducibility is potentially a problem in all scientific disciplines. However, human clinical trials seem to be less at risk because they are already governed by various regulations that stipulate rigorous design and independent oversight — including randomization, blinding, power estimates, pre-registration of outcome measures in standardized, public databases such as ClinicalTrials.gov and oversight by institutional review boards and data safety monitoring boards. Furthermore, the clinical trials community has taken important steps towards adopting standard reporting elements[7].

Challenges to Ensuring Rigor and Transparency in Reporting Science

- Science often viewed as "self-correcting;" immune from reproducibility problems
  - Principle remains true over the long-term
- Checks and balances for reproducibility in the short- and medium-term are hobbled by interrelated factors
  - Results in compromised ability to reproduce findings of others, particularly in preclinical research studies involving animal models of disease

Challenges to Ensuring Rigor and Transparency in Reporting Science: Factors that "Short Circuit" Self-Correction

- Current "hyper-competitive" environment is fueled, in part, by:
  - Historically low funding rates
  - Over-dependence on "high profile" publications when grants are reviewed; institutions are making appointment, promotion, and tenure decisions
- Publication practices that contribute:
  - Difficulty in publishing negative findings
  - Overemphasis on the "exciting, big picture" finding sometimes results in publications leaving out necessary details of experiments

Challenges to Ensuring Rigor and Transparency in Reporting Science: Factors that "Short Circuit" Self-Correction

- Poor training leading to:
  - Inadequate experimental design – fundamental quality characteristics not reported/performed (e.g. blinded assessment, randomization, sample size calculations)
  - Inappropriate use of statistics ("p-hacking")
  - Incomplete reporting of resources used and/or unexpected variability in resources

PERSPECTIVES

CELL BIOLOGY

Fixing problems with cell lines

Technologies and policies can improve authentication

By Jon R. Lorsch[1*], Francis S. Collins[2], Jennifer Lippincott-Schwartz[3,4]

Despite the important role of cell culture in the study of biology and medicine, evidence has accumulated that cell lines are frequently misidentified or contaminated by other cells or microorganisms. This can be a substantial problem in many fields, such as cancer research, where drugs are initially tested using a cell line derived from the targeted type of tumor (*1*). If a drug is tested on the wrong cell line, research can lead to unreliable results, and discovery of effective treatments can be delayed. Even in basic research, use of mistaken cell lines can hinder progress because of variations in cell behavior among different cell types. Given these concerns, developing corrective measures for cell line misidentification and contamination warrants renewed attention.

POLICY

Since the 1960s, more than 400 widely used cell lines worldwide have been shown to have been misidentified (*2*, *3*). Cells originally thought to have been derived from one tissue type have later been found to be from a different tissue. In some cases, even the species of the cells has been misidentified. A 2011 study of 122 different head and neck cancer cell lines revealed that 37 (30%) were misidentified (*4*). Analyses of a variety of tissue culture collections and cells sent to repositories for curation and storage from labs in the United States, Europe, and Asia suggest that at least 15% of cell lines are misidentified or contaminated (*4*, *5*).

Misidentified cell lines can create problems at many levels of biomedical research. For example, studies using just two misidentified cell lines were included in three grants funded by the U.S. National Institutes of Health (NIH), two clinical trials, 11 patents, and >100 papers (*6*). Nonetheless, the need for validation and accurate reporting of cell line identity does not appear to be widely recognized by researchers; a 2013 study found that fewer than half of cell lines were unambiguously identified in published studies (*7*).

A number of factors contribute to the problems of cell line misidentification and contamination. For example, inadvertently using a pipette more than once when working with different cell lines in culture can lead to cross contamination. If the contaminating cell line divides more rapidly than the original cells, it can quickly dominate the population, changing the identity of the culture. This event often goes undetected because cells from dif-

ILLUSTRATION: PETER AND MARIA HOEY/WWW.PETERHOEY.COM

Corrected 19 December, 2014; see full text.

Published by AAAS

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Reproducibility in Cell Culture Studies

Possible action areas:

- Ask applicants for their plans to validate key reagents, including cell lines
- Facilitate the development and dissemination of consensus standards for authentication, handling, controls, and reporting
- Promote development of more efficient and cost-effective tools for characterizing cell lines and reagents
- Promote development of defined, controllable and affordable cell culture media and reagents

COMMENT

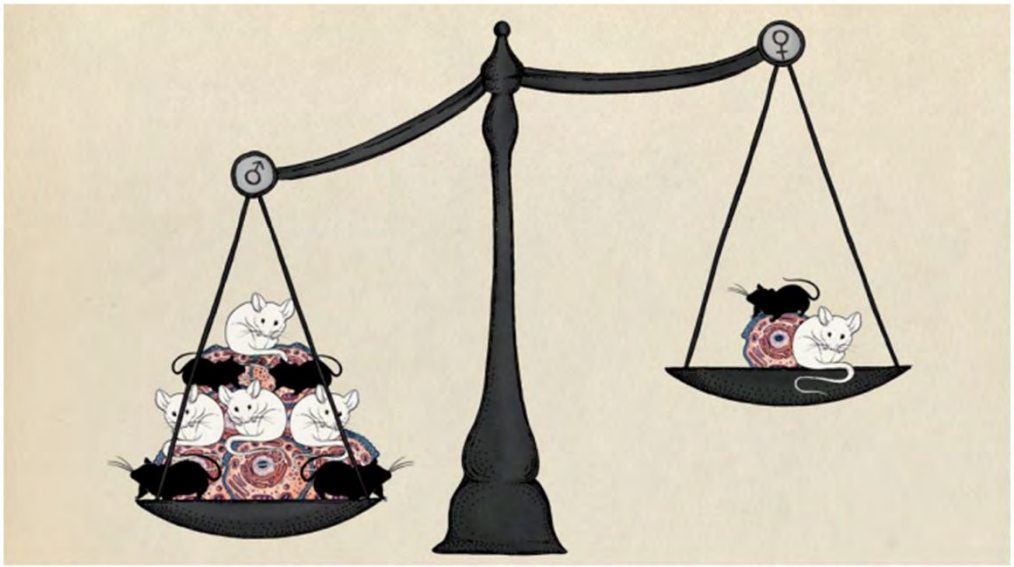


ILLUSTRATION BY KATIE SCOTT

NIH to balance sex in cell and animal studies

Janine A. Clayton and **Francis S. Collins** unveil policies to ensure that preclinical research funded by the US National Institutes of Health considers females and males.

More than two decades ago, the US National Institutes of Health (NIH) established the Office of Research on Women's Health (ORWH). At that time, the Congressional Caucus for Women's Issues, women's health advocacy groups and NIH scientists and leaders agreed that excluding women from clinical research was bad for women and bad for science. In 1993, the NIH Revitalization Act required the inclusion of women in NIH-funded clinical research.

Today, just over half of NIH-funded clinical-research participants are women. We know much more about the role of sex and gender in medicine, such as that low-dose aspirin has different preventive effects in women and men, and that drugs such as calls to action[1]. Publications often continue to neglect sex-based considerations and analyses in preclinical studies[2,3]. Reviewers, for the most part, are not attuned to this failure. The over-reliance on male animals and cells in preclinical research obscures key sex differences that could guide clinical studies. And it might be harmful: women experience higher rates of adverse drug reactions than men do[4]. Furthermore, inadequate inclusion of female cells and animals in experiments and inadequate analysis of data by sex may well contribute to the troubling rise of irreproducibility in preclinical biomedical research, which the NIH is now actively working to address[5,6].

The NIH plans to address the issue of sex and gender inclusion across biomedical research multi-dimen- stakeholders including publishers. This move is essential, potentially very powerful and need not be difficult or costly.

BETTER WITH BOTH

Certain rigorous studies evaluating the effects of sex differences have been effective in bridging the divide between animal and human work. One example concerns multiple sclerosis (MS). Women are more susceptible to MS than men are, but develop less-severe forms of the disease. The most widely accepted MS animal model — rodent experimental autoimmune encephalomyelitis (EAE) — has revealed[7] that sex differences in MS are related to both reproductive and non-reproductive factors. Findings[8] that oestrogen therapy provided benefits in rodent EAE

"Over the course of FY 2015, NIH plans to roll out policies that will require applicants to address inclusion of both sexes in biomedical research."

Factorial Design: Addressing Sex as a Second Independent Variable

Review TRENDS in Pharmacological Sciences Vol.24 No.7 July 2003 341

ELSEVIER

Principles: The need for better experimental design

Michael F.W. Festing

c/o FRAME (Fund for the Replacement of Animals in Medical Experiments), Russell and Burch House, 96–98 North Sherwood Street, Nottingham NG1 4EE, UK

256 *Laboratory Animals* (1992) **26**, 256–267

REVIEW ARTICLE

The scope for improving the design of laboratory animal experiments

MICHAEL F. W. FESTING

MRC Toxicology Unit, Woodmansterne Road, Carshalton, Surrey SM5 4EF, UK

Summary

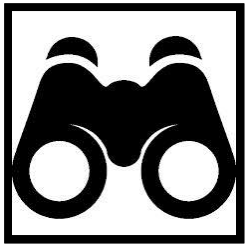
The factors which need to be taken into account in designing a 'good' experiment are reviewed. Such an experiment should be unbiased, have high precision, a wide range of applicability, it animals should be required to take formal training courses which include sessions on experimental design in order to minimize animal use and to increase experimental efficiency.

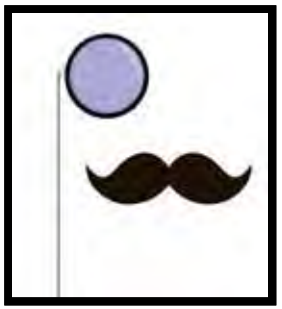
"The importance of variables can often be evaluated efficiently using factorial experimental designs, without any substantial increase in the overall number of animals."

Lab Anim. 1992 Oct;26(4):256-68.; *Exp Gerontol*. 1997 Jan-Apr;32(1-2):39-47.; *Trends Pharmacol Sci*. 2003 Jul;24(7):341-5

NIH National Institutes of Health Office of Extramural Research

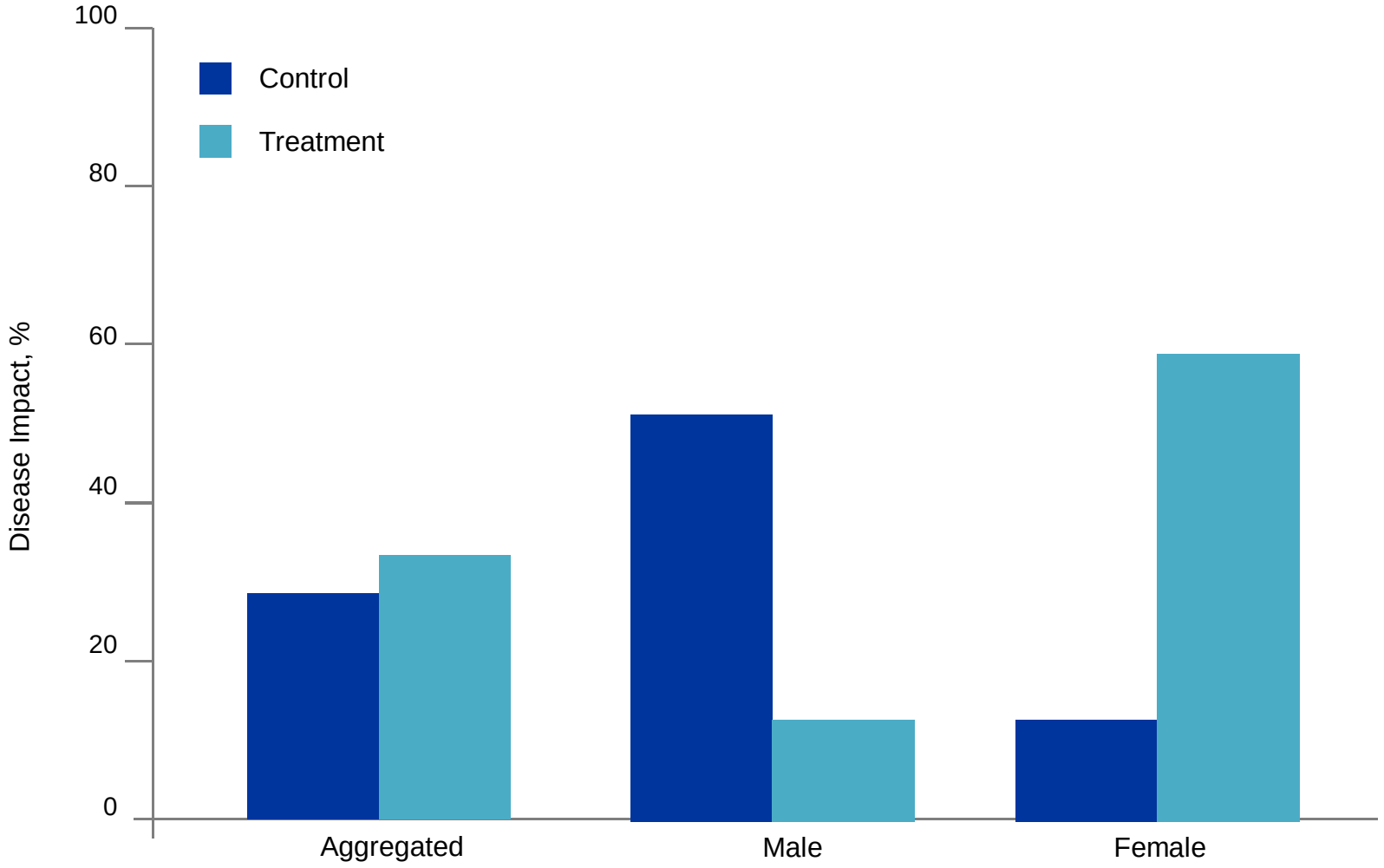
Factorial Experimental Design



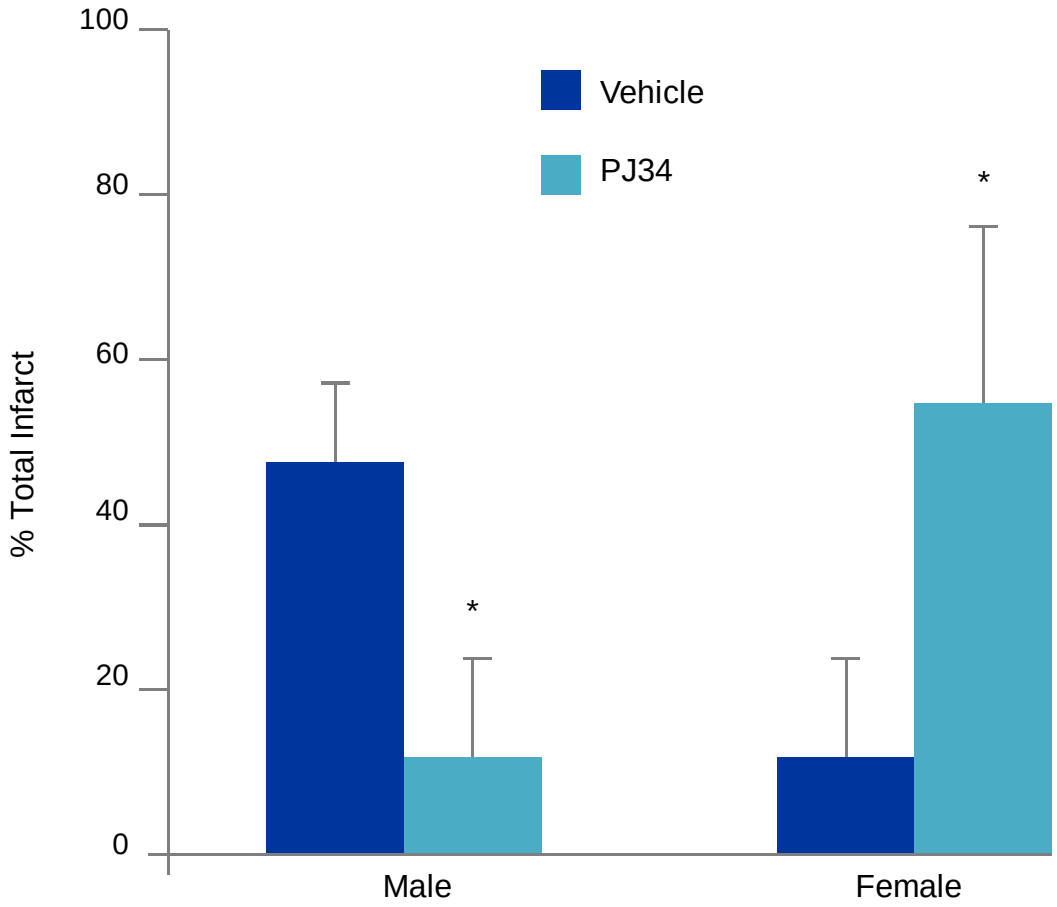


	Male	Female
Control	--	++
Treatment	++	--

Biological/Disease Impact of Experimental Design



Real Life



The effects of the selective poly-ADP ribose polymerase (PARP-1) inhibitor PJ-34 in wild-type (WT) mice of both genders. Treatment with PJ-34 at ischemic onset reduced total infarction in male mice compared with saline-treated controls (* $P<0.001$). A significant increase in ischemic damage was seen in PJ-34-treated females compared with control (* $P<0.001$).

McCullough LD, et al. *Journal of Cerebral Blood Flow & Metabolism* (2005) **25,** 502ï 512.

New Journal Policies to Enhance Reproducibility

Science

EDITORIAL

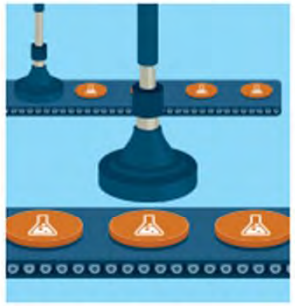
Journals unite for reproducibility

Reproducibility, rigor, transparency, and independent verification are cornerstones of the scientific method. Of course, just because a result is reproducible does not necessarily make it right, and just because it is not reproducible does not necessarily make it wrong. A transparent and rigorous approach, however, can almost always shine a light on issues of reproducibility. This light ensures that science moves forward, through independent verifications as well as the course corrections that come from refutations and the objective examination of the resulting data.

It was with the goal of strengthening such approaches in the biomedical sciences that a group of editors representing over 30 major journals, representatives from funding agencies, and scientific leaders assembled at the AAAS headquarters in June of 2014 to discuss principles and guidelines for preclinical biomedical research. The gathering was convened by the U.S. National Institutes of Health, *Nature*,* and *Science*.

The discussion ranged from what journals were already doing to address reproducibility and the effectiveness of those measures, to the magnitude of the problem and the cost of solutions. The attendees agreed on a common set of Principles and Guidelines in Reporting Preclinical Research (www.nih.gov/about/reporting-preclinical-research.htm) that list proposed journal policies and author reporting requirements to promote transparency and reproducibility.

The new guidelines suggest that journals include in their information for authors their policies for statistical analysis and how they review the statistical accuracy of work under consideration. Any imposed page limits should not discourage reproducibility. The guidelines encourage using a checklist to ensure the reporting of important experimental parameters, such as standards used, number and type of replicates, statistics, method of randomization, whether experimenters were blind to the conduct of the experiment, how the sample size was determined, and what criteria were used to include or exclude any data. Journals should recommend the deposition of data in public repositories where available and link data bidirectionally to the published paper. Journals should strongly encourage, as appropriate, that all materials used in the experiment be shared with those who wish to replicate the experiment. Once a journal publishes a paper, it assumes the obligation to consider publication of a refutation of that paper, subject to its usual standards of quality.



"...scientific journals are standing together in their conviction that reproducibility and transparency are important..."

The more open-ended portion of the guidelines suggests that journals establish best practices for image-based data (such as screening for manipulation and storing full-resolution archival versions) and how to describe experiments more completely. An example for animal experiments is reporting the source, species, strain, sex, age, husbandry, inbred and strain characteristics, or transgenic animals, etc. For cell lines, one might report the source, authentication, and mycoplasma contamination status. The existence of these guidelines does not obviate the need for replication or independent verification of research results, but should make it easier to perform such replication.

Some of the journals at the meeting already had implemented all or most of these principles and guidelines. But the important point is that a large number of scientific journals are standing together in their conviction that reproducibility and transparency are important issues.† As partners to the research enterprise in the communication and dissemination of research results, journals want to do their part to raise the standards for the benefit of all scientists and the benefit of society. The hope is that that these guidelines will not be viewed as onerous, but as part of the quality control that justifies the public trust in science.

–Marcia McNutt

Marcia McNutt
Editor-in-Chief
Science *Journals*

www.nature.com/news/1.16259. † A list of all journals and publishers signatory to the principles and guidelines www.nih.gov/about/reporting-preclinical-research.htm.

10.1126/science.aaa1

nature

EDITORIALS

CONSERVATION Saving species is far from a walk in the park p.8

WORLD VIEW Psychology gears up to check its workings p.9

BREAKFAST Chimps plan days to ensure they nab tastiest figs p.11

Journals unite for reproducibility

Consensus on reporting principles aims to improve quality control in biomedical research and encourage public trust in science.

Reproducibility, rigour, transparency and independent verification are cornerstones of the scientific method. Of course, just because it is not reproducible does not make it wrong. A transparent and rigorous approach, however, will almost always shine a light on issues of reproducibility. This light ensures that science moves forward, through independent verifications as well as the course corrections that come from refutations and the objective examination of the resulting data.

It was with the goal of strengthening such approaches in the biomedical sciences that a group of editors representing more than 30 major journals; representatives from funding agencies; and scientific leaders assembled at the American Association for the Advancement of Science's headquarters in June 2014 to discuss principles and guidelines for preclinical biomedical research. The gathering was convened by the US National Institutes of Health, *Nature* and *Science* (see *Science* **346**, 679; 2014).

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Principles and Guidelines for Reporting Preclinical Research

- Rigorous statistical analysis
- Transparency in reporting
- Data and material sharing
- Consideration of refutations
- Consider establishing best practice guidelines for:
  - Antibodies
  - Cell lines
  - Animals

- Standards
- Replicates
- Statistics
- Randomization
- Blinding
- Sample size estimation
- Inclusion/exclusion criteria

http://www.nih.gov/about/reporting-preclinical-research.htm

Clearinghouse for Trainin ×

www.nigms.nih.gov/training/pages/clearinghouse-for-training-modules-to-enhance-data-reproducibility.aspx

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Clearinghouse for Training Modules to Enhance Data Reproducibility

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Related Information

NIH Reproducibility Workshops on Modern Technologies: Potentials and Pitfalls
- Cell Biology
- Structural Biology

In January 2014, NIH launched a series of initiatives to enhance rigor and reproducibility in research. As a part of this initiative, NIGMS, along with nine other NIH institutes and centers, issued the funding opportunity announcement RFA-GM-15-006 to develop, pilot and disseminate training modules to enhance data reproducibility. Graduate students, postdoctoral fellows and early stage investigators are the primary audiences for these training modules.

For the benefit of the scientific community, we will be posting the products of these grants on this Web site as they become available in the future.

In addition, we are sharing here a series of four training modules developed by NIH. These modules focus on integral aspects of rigor and reproducibility in the research endeavor, such as bias, blinding and exclusion criteria. The modules are not meant to be comprehensive, but rather are intended as a foundation to build on and a way to stimulate conversations, which may be facilitated by the use of the accompanying discussion materials. Currently, the modules are being integrated into NIH intramural training activities.

NIH Rigor and Reproducibility Training Modules

Introduction to the Modules [PDF, 110KB]

Module 1: Lack of Transparency
In order to reproduce someone else's findings adequately, the experimental methods, rationale and other pertinent information must be accessible and understandable. This module highlights the need to include all relevant details in publications to ensure that other studies are able to build upon the research appropriately and accurately.
Lack of Transparency Discussion Material [PDF, 97.2KB]

Module 2: Blinding and Randomization
Sample blinding and randomization are key elements in reducing selection and other biases as well as in permitting reliable statistical testing. This module presents the importance of blinding and randomization, as well as the impact of issues that may introduce bias, such as pressure to publish.
Blinding and Randomization Discussion Material [PDF, 104KB]

Module 3: Biological and Technical Replicates
Including replicates in the experimental process is essential to ensuring the most rigorous research approach. In this module, reviewers discuss a figure included in a grant application and the potential significance of the finding, which leads to a brief conversation about the differences between biological and technical replicates.

Trans – NIH Pilots

Pilot Focus	Types of Efforts Being Developed
Evaluation of scientific premise in grant applications	New Funding Opportunities with additional review criteria regarding scientific premise
Checklist and Reporting Guidelines	Reviewer checklists regarding reporting standards and scientific rigor
Changes to Biosketch	Biosketch pilot with focus on accomplishments and not just publications
Approaches to reduce "perverse incentivesòto publish	Exploring award options with a longer period of support for investigators
Supporting replication studies	New Funding Opportunities for replication studies, and options to assess whether pre-clinical findings should be replicated
Training	Developing materials on experimental design
Other efforts	Use of Prize Challenges to encourage reproducibility of results, PubMed Commons

Our Guiding Principles for Rigor & Transparency

- *Clarify* NIH's long-standing expectations regarding rigor and transparency and how we would like to see this described in applications
- Raise awareness and begin culture shifts in the scientific community
- Prompt applicants to consider issues that they may have previously down-played or ignored, which may have a detrimental effect on the quality of the science they produce

Our Guiding Principles for Rigor & Transparency

- Improve the way that applicants describe their work; provide sufficient information for reviewers
- Demonstrate to our public stakeholders that NIH is seriously considering their concerns
- As always, ensure that NIH is investing in the best science and minimizing unnecessary burden

Rock Talk

Helping connect you with the NIH perspective

Posted on June 9, 2015 by Sally Rockey and Larry Tabak

Enhancing Reproducibility in NIH-supported Research through Rigor and Transparency

Dr. Larry Tabak is the Principal Deputy Director of NIH.

Nothing could be more important to our enterprise than research rigor, assuring that the results of our work are reproducible. Our conversation with you on this topic began early last year with a commentary in Nature by Francis Collins and today's guest blogger, Larry Tabak, on the importance of reproducibility and how NIH plans to enhance it. As described in a follow-up Rock Talk post, the topic of reproducibility is not new. Evidence has shown that too many biomedical-research publications are irreproducible. Thus this topic demanded our community's immediate attention and we have had continued dialog with and participation by you over the course of the last 18 months to describe the issue, request information, launch

Dr. Sally Rockey is NIH's Deputy Director for Extramural Research, serving as the principal scientific leader and advisor to the NIH Director on the NIH extramural research program.

Search

Blog Policies

Learn More

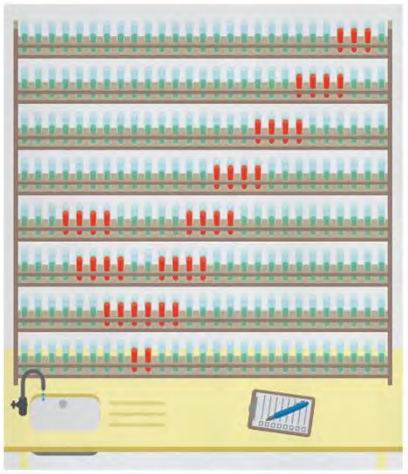
- NIH Grants Website
- Office of Extramural

Learning Objectives

- Describe the issue of reproducibility and NIH plans to address it
- Summarize changes to application instructions and review criteria for NIH grants
- Explain how the policies behind rigor and transparency will impact different types of grants along with the implementation timeline

Four Areas of Clarification

- Scientific premise
- Scientific rigor
- Relevant Biological Variables, Such as Sex
- Authentication of Key Biological and/or Chemical Resources



outnumbered by the hundreds of thousands published each year in good faith.

Instead, a complex array of other factors seems to have contributed to the lack of reproducibility. Factors include poor training of researchers in experimental design; increased emphasis on making provocative statements rather than presenting technical details; and publications that do not report basic elemer
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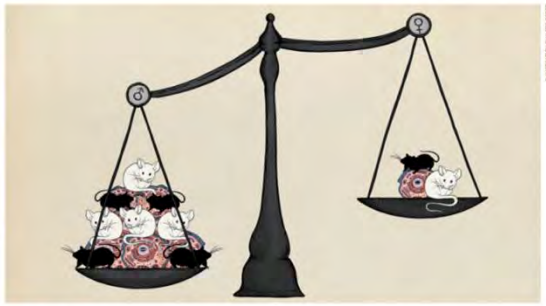
COMMENT

NIH plans to enhance reproducibility

Francis S. Collins and **Lawrence A. Tabak** discuss initiatives that the US National Institutes of Health is exploring to restore the self-correcting nature of preclinical research.



NIH to balance sex in cell and animal studies

Janine A. Clayton and **Francis S. Collins** unveil policies to ensure that preclinical research funded by the US National Institutes of Health considers females and males.

A growing chorus of concern, from scientists and laypeople, contends that the complex system for ensuring

PERSPECTIVES

CELL BIOLOGY

Fixing problems with cell lines

Technologies and policies can improve authentication

By Jon R. Lorsch[*], Francis S. Collins[†], Jennifer Lippincott-Schwartz[‡]

Despite the important role of cell culture in the study of biology and medicine, evidence has accumulated that cell lines are frequently misidentified or contaminated by other cells or microorganisms. This can be a substantial problem in many fields, such as cancer research, where drugs are initially tested using a cell line

POLICY derived from the targeted type of tumor (1). If a drug is tested on the wrong cell line, research can lead to unreliable results, and discovery of effective treatments can be delayed. Even in basic research, use of mistaken cell lines can hinder progress because of variations in cell behavior among different cell types. Given these concerns, developing corrective measures for cell line misidentification and contamination warrants renewed attention.

Since the 1960s, more than 400 widely used cell lines worldwide have been shown to have been misidentified (2, 3). Cells originally thought to have been derived from one tissue type have later been found to be from a different tissue. In some cases, even the species of the cells has been misidentified. A 2011 study of 122 different head and neck cancer cell lines revealed that 37 (30%) were misidentified (4). Analyses of a variety of tissue culture collections and cells sent to repositories for curation and storage from labs in the United States, Europe, and Asia suggest that at least 15% of cell lines are misidentified or contaminated (4, 5).

Misidentified cell lines can create problems at many levels of biomedical research. For example, studies using just two misidentified cell lines were included in three grants funded by the U.S. National Institutes of Health (NIH), two clinical trials, 11 patents, and >100 papers (6). Nonetheless, the need for validation and accurate reporting of cell line identity does not appear to be widely recognized by researchers; a 2013 study found that fewer than half of cell lines were unambiguously identified in published studies (7).

A number of factors contribute to the problems of cell line misidentification and contamination. For example, inadvertently using a pipette more than once when working with different cell lines in culture can lead to cross contamination. If the contaminating cell line divides more rapidly than the original cells, it can quickly dominate the population, changing the identity of the culture. This event often goes undetected because cells from dis-

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action. Publications often continue to sex-based considerations and analyses clinical studies. Reviewers, for the art, are not attuned to this failure. The eliance on male animals and cells in ical research obscures key sex differ- hat could guide clinical studies. And it be harmful: women experience higher f adverse drug reactions than men do. rmore, inadequate inclusion of female d animals in experiments and inade- analysis of data by sex may well contrib- the troubling rise of irreproducibility linical biomedical research, which the now actively working to address. NIH plans to address the issue of gender inclusion across biomedical h multi-dimen-

stakeholders including publishers. This move is essential, potentially very powerful and need not be difficult or costly.

BETTER WITH BOTH

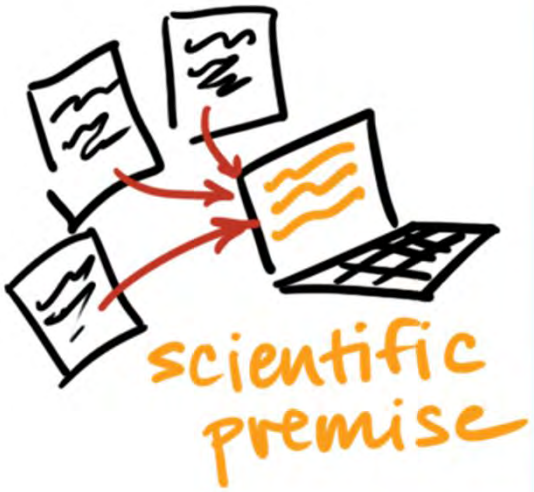
Certain rigorous studies evaluating the effects of sex differences have been effective in bridging the divide between animal and human work. One example concerns multiple sclerosis (MS). Women are more susceptible to MS than men are, but develop less-severe forms of the disease. The most widely accepted MS animal model — rodent experimental autoimmune encephalomyelitis (EAE) — has revealed that sex differences in MS are related to both reproductive and non-reproductive factors. Findings that oestrogen therapy provided benefits in rodent EAE

NIH National Institutes of Health
Office of Extramural Research

RPG Application and Review

Element of Rigor	Section of Application	Criterion Score	Additional Review Consideration	Contribute to Overall Impact?
Scientific Premise		Significance	NA	Yes
Scientific Rigor	Research Strategy	Approach	NA	Yes
Consideration of Sex and Other Relevant Biological Variables		Approach	NA	Yes
Authentication of Key Biological and/or Chemical Resources	New Attachment	NA	Acceptable or unacceptable	No

Scientific Premise



- **All research builds upon prior research**, whether observations, preliminary data, or published literature. The scientific premise for an application is the research that is used to form the basis for the proposed research question.

Å **NIH expects applicants to describe the general strengths and weaknesses of the prior research being cited by the applicant as crucial to support the application.** It is expected that this consideration of general strengths and weaknesses could include attention to the rigor of the previous experimental designs, as well as the incorporation of relevant biological variables and authentication of key resources.

Scientific Premise
Research Plan Instructions - Significance

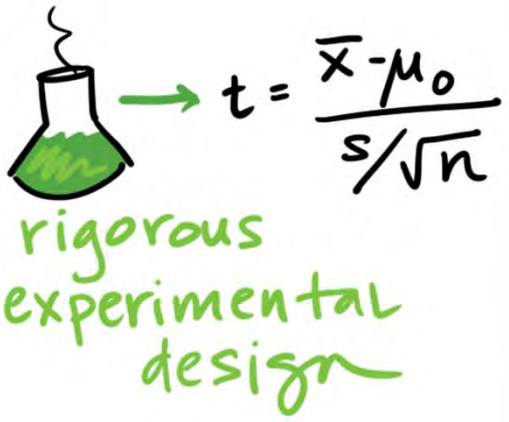
- Explain the importance of the problem or critical barrier to progress in the field that the proposed project addresses.
- *Describe the scientific premise for the proposed project, including consideration of the strengths and weaknesses of published research or preliminary data crucial to the support of your application.*
- Explain how the proposed project will improve scientific knowledge, technical capability, and/or clinical practice in one or more broad fields.
- Describe how the concepts, methods, technologies, treatments, services, or preventative interventions that drive this field will be changed if the proposed aims are achieved.

Scientific Premise
Significance – Review Questions

- Does the project address an important problem or a critical barrier to progress in the field?
- *Is there a strong scientific premise for the project?*
- If the aims of the project are achieved, how will scientific knowledge, technical capability, and/or clinical practice be improved?
- How will successful completion of the aims change the concepts, methods, technologies, treatments, services, or preventative interventions that drive this field?

Scientific Rigor

- **Scientific rigor** is the strict application of the scientific method to ensure robust and unbiased experimental design, methodology, analysis, interpretation and reporting of results.



Å NIH expects applicants to describe the experimental design and methods proposed and how they will achieve robust and unbiased results. Robust and unbiased results are obtained using methods designed to avoid bias and these results can be reproduced under well-controlled and reported experimental conditions.

Scientific Rigor
Research Plan Instructions - Approach

- Describe the overall strategy, methodology, and analyses to be used to accomplish the specific aims of the project. *Describe the experimental design and methods proposed and how they will achieve robust and unbiased results.* Unless addressed separately in the Resource Sharing Plan attachment below, include how the data will be collected, analyzed, and interpreted.
- Discuss potential problems, alternative strategies, and benchmarks for success anticipated to achieve the aims.
- If the project is in the early stages of development, describe any strategy to establish feasibility, and address the management of any high risk aspects of the proposed work.

Scientific Rigor
Approach – Review Questions

- Are the overall strategy, methodology, and analyses well-reasoned and appropriate to accomplish the specific aims of the project?
- *Have the investigators presented strategies to ensure a robust and unbiased approach, as appropriate for the work proposed?*
- Are potential problems, alternative strategies, and benchmarks for success presented?
- If the project is in the early stages of development, will the strategy establish feasibility and will particularly risky aspects be managed?

Consideration of Relevant Biological Variables, Such as Sex

- Biological variables, such as sex, age, weight, and underlying health conditions, are often critical factors affecting health or disease.

Å NIH expects that sex as a biological variable will be factored into research designs, analyses, and reporting in vertebrate animal and human studies. Strong justification from the scientific literature, preliminary data or other relevant considerations must be provided for applications proposing to study only one sex.

Relevant Biological Variables Research Plan Instructions – Approach

- *Explain how relevant biological variables, such as sex, are factored into research designs and analyses for studies in vertebrate animals and humans.*
  - *For example, strong justification from the scientific literature, preliminary data, or other relevant considerations, must be provided for applications proposing to study only one sex.*
  - *Please refer to NOT-OD-XXX for further consideration of NIH expectations about sex as a biological variable.*

Relevant Biological Variables Approach – Review Questions

- *Have the investigators presented adequate plans to address relevant biological variables, such as sex, for studies in vertebrate animals or human subjects?.*

Authentication of Key Biological and/or Chemical Resources

- The quality of the resources used to conduct research is critical to the ability to reproduce the results. NIH expects that key biological and/or chemical resources will be regularly authenticated to ensure their identity and validity for use in the proposed studies.

Å Key biological and/or chemical resources are those that: 1) may differ from laboratory to laboratory or over time; 2) may have qualities and/or qualifications that could influence the research data; and 3) are integral to the proposed research and may or may not be generated with NIH funds. **These include, but are not limited to, cell lines, specialty chemicals, antibodies and other biologics.**

Authentication of Key Resources
Other Research Plan Sections - Instructions

Briefly describe methods to ensure the identity and validity of key biological and/or chemical resources used in the proposed studies.

- *Key biological and/or chemical resources may or may not be generated with NIH funds and:*
  - *1) may differ from laboratory to laboratory or over time;*
  - *2) may have qualities and/or qualifications that could influence the research data; and*
  - *3) are integral to the proposed research. These include, but are not limited to, cell lines, specialty chemicals, antibodies, and other biologics.*
- *Standard laboratory reagents that are not expected to vary do not need to be included in the plan. Examples are buffers and other common biologicals or chemicals.*
- *Reviewers will assess the information provided in this Section. Any reviewer questions associated with key biological and/or chemical resource authentication will need to be addressed prior to award.*

Authentication of Key Resources Additional Review Consideration

- *For projects involving key biological and/or chemical resources, reviewers will comment on the brief plans proposed for identifying and ensuring the validity of those resources.*

Learning Objectives

- Describe the issue of reproducibility and NIH plans to address it
- Summarize changes to application instructions and review criteria for NIH grants
- Explain how the policies behind rigor and transparency will impact different types of grants along with the implementation timeline

NIH Funds Many Types of Grants: Does Rigor Policy Apply to All?

- Administrative supplements
- Career Development
- Centers
- Conferences
- Construction
- Fellowships
- Instrumentation
- Program Projects
- Publication support
- Research
- Resource & Resource Related
- Small Business
- Training