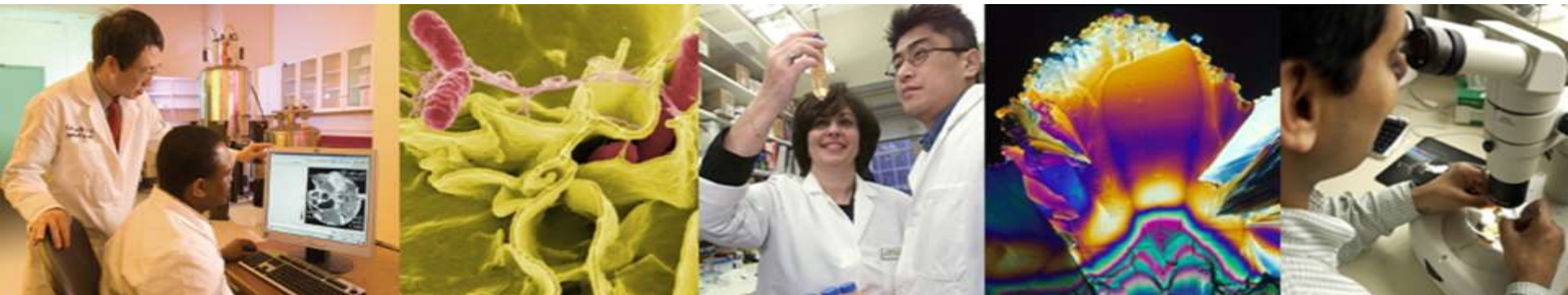


On the Reproducibility of Biomedical Research

Sixth Annual Lecture on Biomedical Ethics

April 15, 2016



Lawrence A. Tabak, DDS, PhD
Principal Deputy Director, NIH
Department of Health and Human Services



Reproducibility



No, not that kind of reproducibility...

The Growing Challenge

- Noted by research community and beyond in several publications
 - Across research areas
 - Especially in preclinical research

PERSPECTIVE JBMR®

Reproducibility of Results in Preclinical Studies: A Perspective From the Bone Field

Stavros C Manolagas¹ and Henry M Kronenberg²

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.

RESEARCH ARTICLE

PSYCHOLOGY

Estimating the reproducibility of psychological science

Open Science Collaboration^{1,2}

The Economist

World politics Business & finance Economics 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

Like 11k Tweet 1,227

Science

Evaluating replicability of laboratory experiments in economics

Colin F. Camerer,^{1,2,3} Anna Dreber,⁴ Eskil Forsell,^{5,6} Teck-Hua Ho,^{7,8,9} Jürgen Huber,¹⁰ Magnus Johannesson,¹¹ Michael Kirchler,^{12,13} Johan Almenberg,¹⁴ Adam Altmeld,¹⁵ Taizhan Chan,¹⁶ Emma Heikensten,¹⁷ Felix Holzmeister,¹⁸ Taisuke Imai,¹⁹ Siri Isaksson,²⁰ Gideon Nave,²¹ Thomas Pfeiffer,^{22,23} Michael Raza,²⁴ Hang Wu²⁵

THE WALL STREET JOURNAL

OPINION | COMMENTARY

Getting the Bogus Studies Out of Science

Government funding should provide more incentives for replicating research

By ADAM MARCUS AND IVAN ORANSKY

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.

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

Florian Prinz, Thomas Schlange and Khusru Asadullah

TECHNICAL COMMENT

PSYCHOLOGY

Comment on "Estimating the reproducibility of psychological science"

Daniel T. Gilbert,^{1,2} Gary King,³ Stephen Pettigrew,⁴ Timothy D. Wilson⁵

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.

Reforming Science: Methodological and Cultural Reforms

Science is “self-correcting”

“In experimental philosophy we are to look upon propositions inferred by general induction from phenomena as accurately or very nearly true...till such time as other phenomena occur, by which they may either be made more accurate, or liable to exception.”

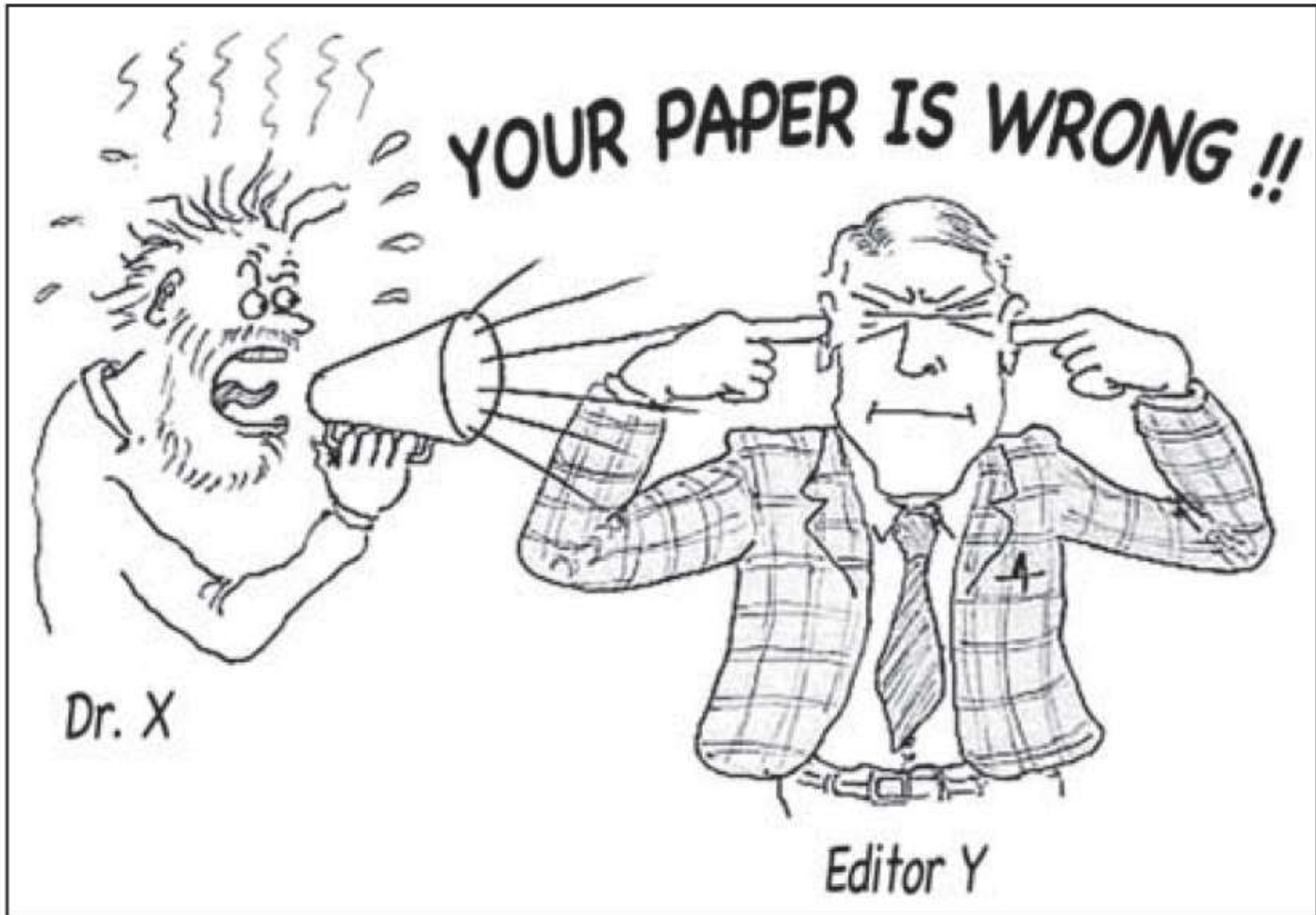
Isaac Newton, *Mathematical Principles of Natural Philosophy*

Science is “self-correcting”

“the really valuable part of the Fourth Rule is that which implies that a *constant verification*, and, if necessary, rectification, of truths discovered by induction, should go on in the scientific world. Even when the law is, or appears to be, most certainly exact and universal, it should be constantly exhibited to us afresh in the form of experience and observation.”

William Whewell, *On the Philosophy of Discovery*

Science is “self-correcting”



So what has gone awry?

Challenges to Ensuring Rigor and Transparency in Reporting Science: Underlying Issues

Publish or perish!

Need for grant support

Misuse of impact factors

Your work must be “novel”

Misaligned Incentives

No negative data

Poor training



Challenges to Ensuring Rigor and Transparency in Reporting Science: Underlying Issues

Publish or perish!

Need for grant support

Misuse of impact factors

Your work must be “novel”

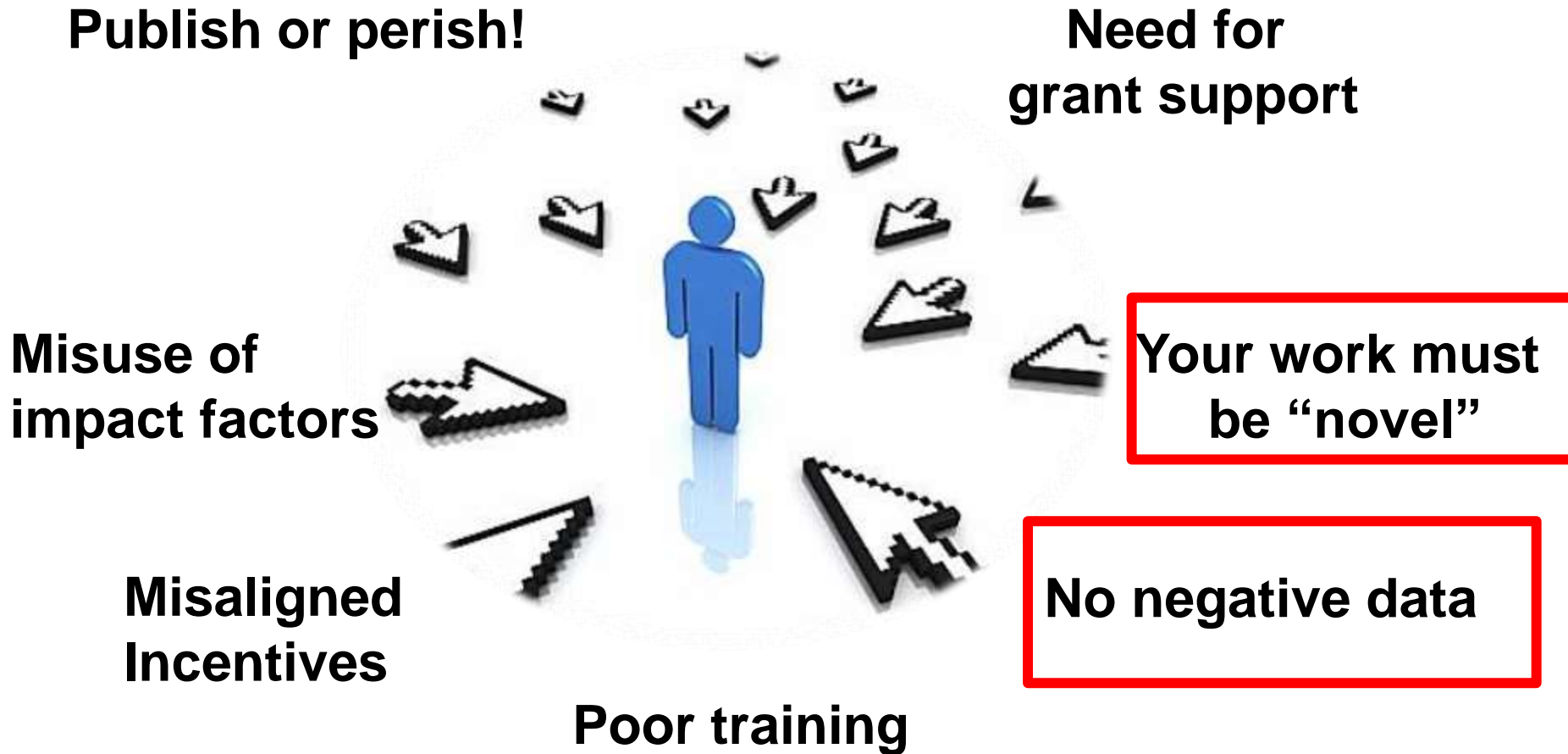
Misaligned Incentives

No negative data

Poor training



Challenges to Ensuring Rigor and Transparency in Reporting Science: Underlying Issues



Challenges to Ensuring Rigor and Transparency in Reporting Science: Underlying Issues



Challenges to Ensuring Rigor and Transparency in Reporting Science: Underlying Issues



Deficiencies in Experimental Procedures

- Insufficient Reporting in publications – blinding, replication & randomization, sample size outliers and exclusion criteria



Insufficient Reporting of Methodological Approaches is Evident for Pre-Clinical Studies

Table 3. Prevalence of selected quality characteristics in other experimental models

	Number of publications	Randomisation (%)	Blinded assessment of outcome (%)	Sample-size calculation (%)
Transgenic stroke studies	157	n/a	3	0
Stroke pathophysiology studies	166	5	18	0
Parkinson's disease	118	12	15	0
Multiple sclerosis	183	2	11	0

Trends Neurosci 2007; 30: 433-439

Design, power, and interpretation of studies in the standard murine model of ALS

Sean Scott, Janice E. Kranz, Jeff Cole, John M. Lincecum, Kenneth Thompson, Nancy Kelly, Alan Bostrom, Jill Theodoss, Bashar M. Al-Nakhala, Fernando G. Vieira, Jeyanthi Ramasubbu & James A. Heywood

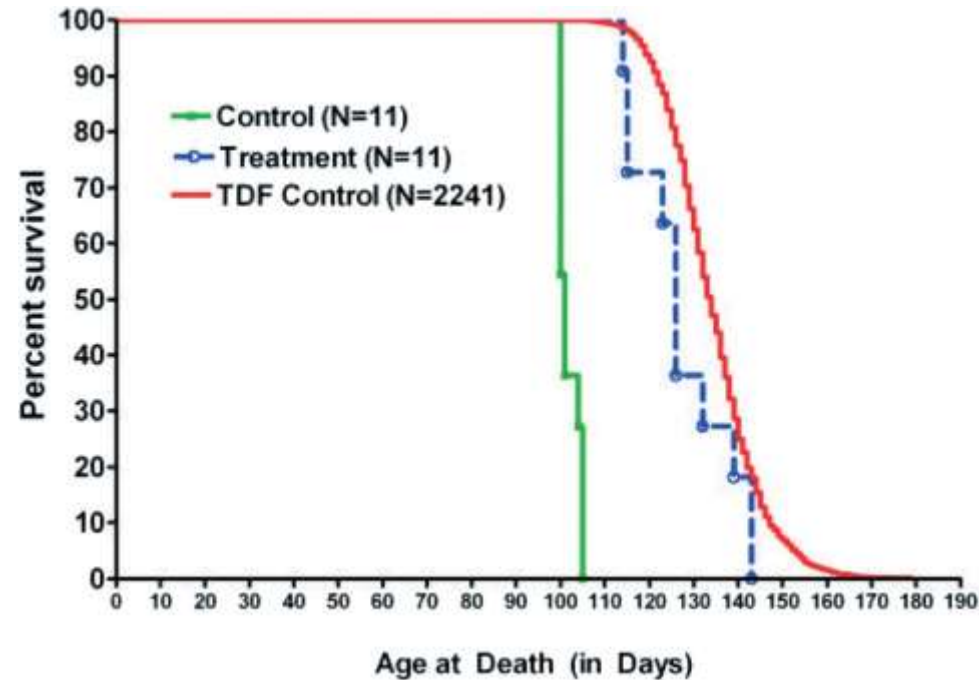


Figure 4. Survival analysis. Control and treated SOD1^{G93A} mice from one publication compared to all of our 2241 control animals (acquired over four years – data from Table S2) that died of ALS.

Deficiencies in Experimental Procedures (cont.)

- Insufficient Reporting in publications – blinding, replication & randomization, sample size outliers and exclusion criteria
- “P-Hacking”

1521-0103/35 1/1/200–205\$25.00
THE JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS
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<http://dx.doi.org/10.1124/jpet.114.219170>
J Pharmacol Exp Ther 351:200–205, October 2014

Commentary

Common Misconceptions about Data Analysis and Statistics

Harvey J. Motulsky

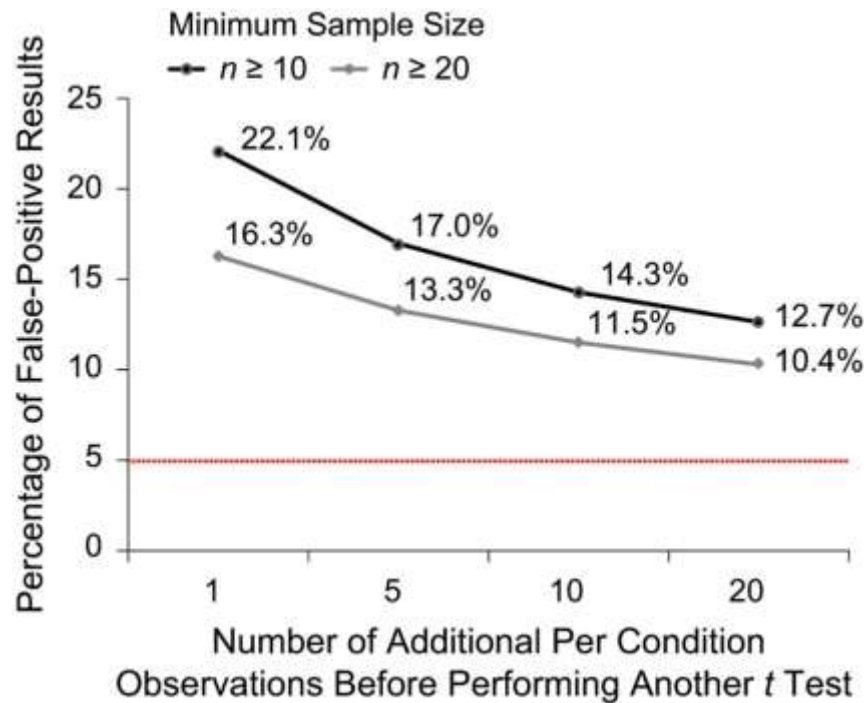
GraphPad Software Inc., La Jolla, California

Received August 8, 2014; accepted August 8, 2014

1) P-hacking, which is when you reanalyze a data set in many different ways, or perhaps reanalyze with additional replicates, until you get the results you want; 2) overemphasis on *P* values rather than on the actual size of the observed effect; 3) overuse of statistical hypothesis testing, and being seduced by the word “significant”; and 4) over-reliance on standard errors, which are often misunderstood.

Deficiencies in Experimental Procedures (cont.)

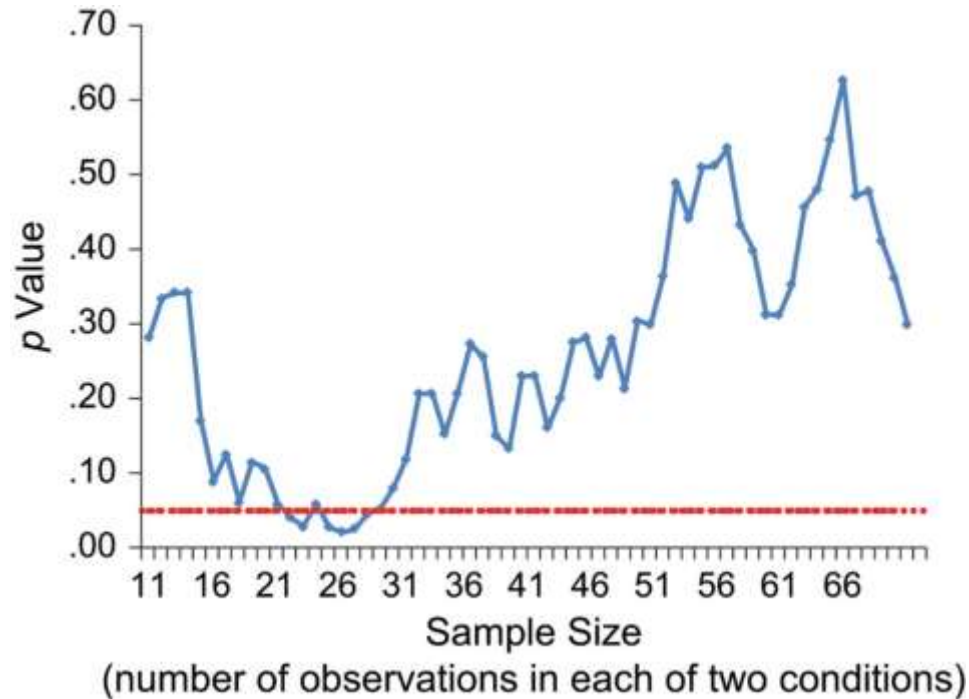
Researcher's "Degrees of Freedom"



Likelihood of obtaining a false-positive result when data collection ends upon obtaining significance ($p \leq .05$, highlighted by the dotted line).

Deficiencies in Experimental Procedures (cont.)

Researcher's "Degrees of Freedom"



Simulation of p values obtained by a researcher who continuously adds an observation to each of two conditions, conducting a t test after each addition

More isn't always better!

Deficiencies in Experimental Procedures (cont.)

Researcher's "Degrees of Freedom"

Simmons et al.

Table 2. Simple Solution to the Problem of False-Positive Publications

Requirements for authors

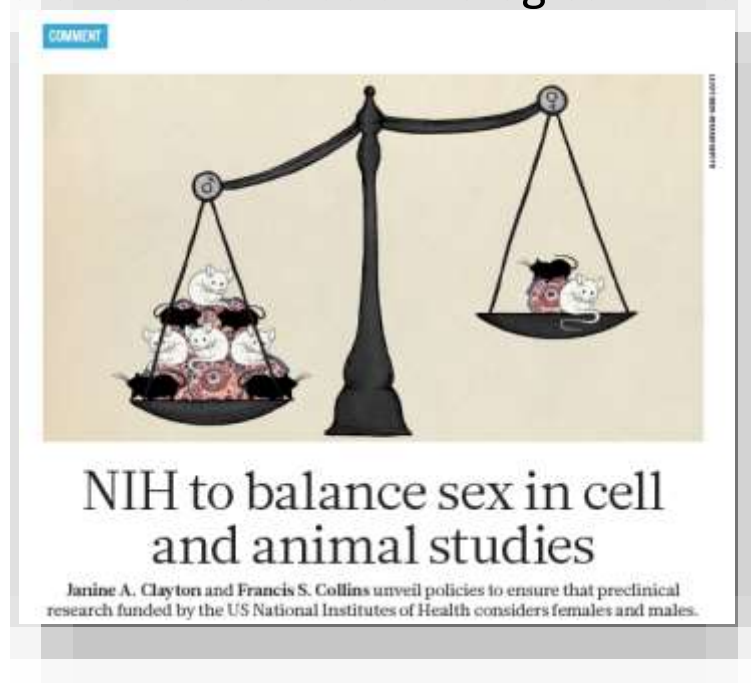
1. Authors must decide the rule for terminating data collection before data collection begins and report this rule in the article.
2. Authors must collect at least 20 observations per cell or else provide a compelling cost-of-data-collection justification.
3. Authors must list all variables collected in a study.
4. Authors must report all experimental conditions, including failed manipulations.
5. If observations are eliminated, authors must also report what the statistical results are if those observations are included.
6. If an analysis includes a covariate, authors must report the statistical results of the analysis without the covariate.

Guidelines for reviewers

1. Reviewers should ensure that authors follow the requirements.
 2. Reviewers should be more tolerant of imperfections in results.
 3. Reviewers should require authors to demonstrate that their results do not hinge on arbitrary analytic decisions.
 4. If justifications of data collection or analysis are not compelling, reviewers should require the authors to conduct an exact replication.
-

Deficiencies in Experimental Procedures (cont.)

- Insufficient Reporting in publications – blinding, replication & randomization, sample size outliers and exclusion criteria
- “P-Hacking”
- Researcher’s “Degrees of Freedom”
- Lack of Consideration of Sex as a Biological Variable



Biological/Disease Impact of Experimental Design

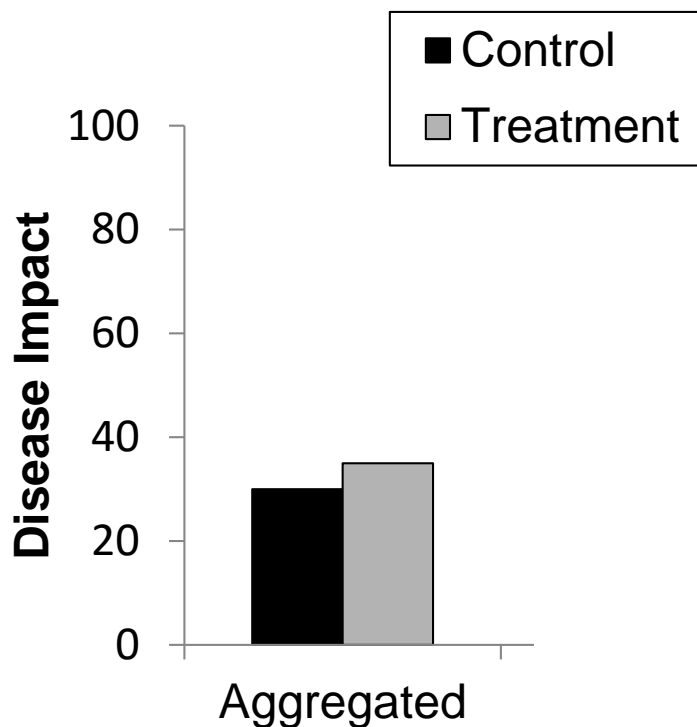
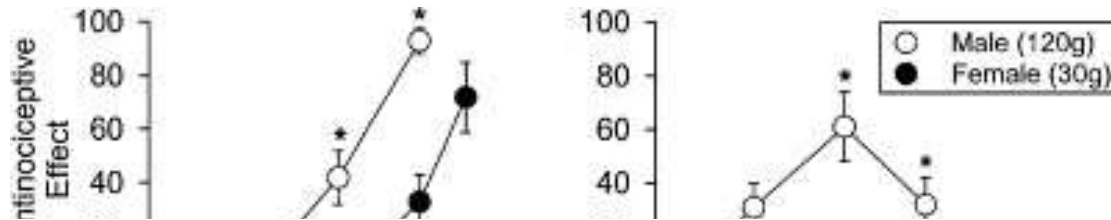


Figure 9 The effects of the selective poly-ADP ribose polymerase (PARP-1) inhibitor PJ-34 in wild-type (WT) mice of both genders. Treatment with PJ-34 at ischemic onset reduced total infarction in male mice compared with saline-treated controls ($*P < 0.001$). A significant increase in ischemic damage was seen in PJ-34-treated females compared with control ($*P < 0.001$).

Importance of Sex as a Biological Variable



1521-0081/68/2/242-263\$25.00
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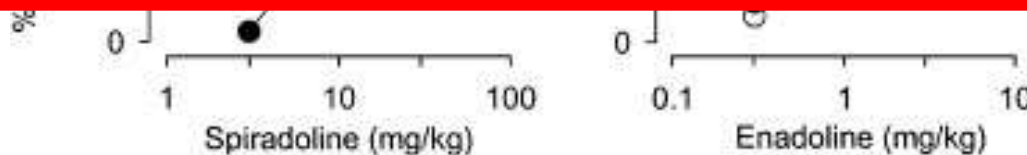
<http://dx.doi.org/10.1124/pr.115.011163>
 Pharmacol Rev 68:242-263, April 2016

ASSOCIATE EDITOR: MICHAEL M. GOTTESMAN

Sex Differences in Animal Models: Focus on Addiction

Jill B. Becker¹ and George F. Koob¹

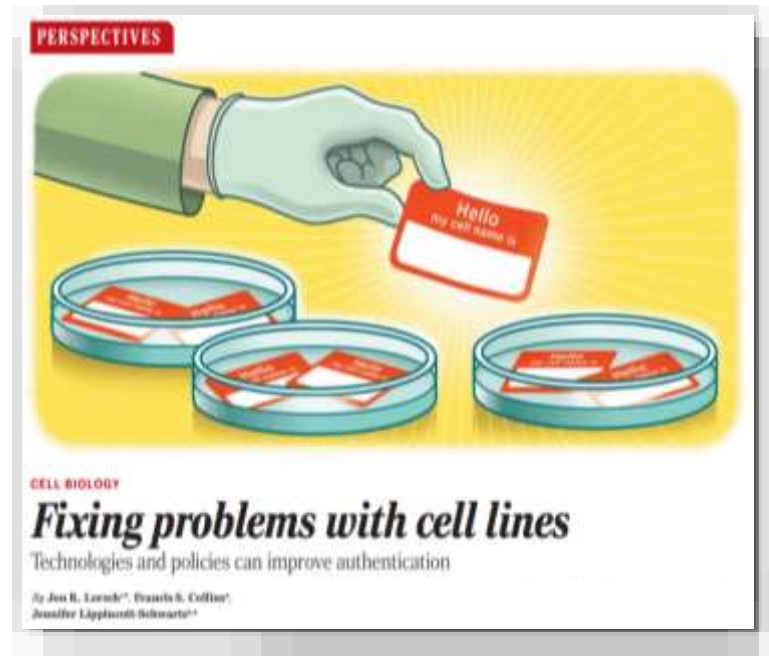
Molecular & Behavioral Neuroscience Institute, Department of Psychiatry, Department of Psychology, University of Michigan, Ann Arbor, Michigan (J.B.B.); and Director, National Institute on Alcohol Abuse and Alcoholism, National Institutes of Health, Bethesda, Maryland (G.F.K.)



Morphine was 2.3-fold more potent in males and buprenorphine produced a 61% effect in males and only a 5% effect in females

Deficiencies in Experimental Procedures (cont.)

- Insufficient Reporting in publications – blinding, replication & randomization, sample size outliers and exclusion criteria
- “P-Hacking”
- Lack of Consideration of Sex as a Biological Variable
- Problems with Authentication of Cell Lines



Reproducibility in Cell Culture Studies

- >400 misidentified cell lines have been cataloged, dating back to the 1960s
- ~70% of researchers surveyed in 2004 had never checked the identity of their cell lines
- Major repositories report that 14-30% of cell lines submitted are contaminated
- In a 2013 survey <50% of cell lines had an unambiguous identifier and source in publications
- Standards for cell line authentication and affordable methods for cell authentication now available

Reproducibility in Cell Culture Studies

New Results

Assessing the prevalence of mycoplasma contamination in cell culture via a survey of NCBI's RNA-seq archive

Anthony O Olarerin-George, John B Hogenesch

doi: <http://dx.doi.org/10.1101/007054>

Abstract

Info/History

Metrics

Data Supplements

Preview PDF

ABSTRACT

Mycoplasmas are notorious contaminants of cell culture and can have profound effects on host cell biology by depriving cells of nutrients and inducing global changes in gene expression. Because they are small, they can escape filtration in culture media. Because they lack cell walls, they are resistant to commonly used antibiotics. Over the last two decades, sentinel testing has revealed wide-ranging contamination rates in mammalian culture. To obtain an unbiased assessment from hundreds of labs, we analyzed sequence data from 9395 rodent and primate

We found 11% of these series were contaminated

bias against mycoplasma detection, had comparable contamination rates as non-poly(A)-selected series. We also examined the relationship between mycoplasma contamination and host gene expression in a single cell RNA-seq dataset and found 61 host genes ($P < 0.001$) were significantly associated with mycoplasma-mapped read counts. Lastly, to estimate the potential economic cost of this widespread contamination, we queried NIH RePORTER to find grants with the terms "cell culture" or "cell lines". Funding for these totaled over \$3 billion, suggesting hundreds of millions of dollars in research are potentially affected. In all, this study suggests mycoplasma contamination is still prevalent today and poses substantial risk to research quality, with considerable financial consequences.

Importance of Cell Line Authentication

Primary tissue	Short tandem repeat locus								TP53 mutation
	D21S11	TH01	D3S1358	FGA	TPOX	D8S1179	vWA	D5S818	
SK-GT-2	29, 32.2	8, 9	15, 17	25, 26	9, 12	13, 15	15, 18	10, 12	c.524G>A
SK-GT-5	28, 32.2	9	15, 17	21, 22	7, 8	10, 13	17	12	c.916C>T

Esophageal Adenocarcinoma cell line (EAC), SK-GT-5, is in fact the gastric fundus carcinoma cell line SK-GT-2!

- More than 100 scientific publications using SK-GT-5 or two other misidentified EAC cell lines have been identified
- Almost half of these reports were based solely on the use of cell lines not representative for EAC

Principles for Addressing Underlying Issues

- Raise community awareness
- Enhance formal training
- Protect the quality of funded and published research by adoption of more systematic review processes
- Share information/data
- Increase stability for investigators

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Raise Community Awareness

NIH Rigor and Reproducibility Web-portal

U.S. Department of Health & Human Services

NIH National Institutes of Health
Turning Discovery Into Health

Search NIH

NIH Employee Intranet | Staff Directory | En Español

Health Information | Grants & Funding | News & Events | Research & Training | Institutes at NIH | About NIH

Home » Research & Training

RIGOR AND REPRODUCIBILITY

Rigor and Reproducibility

- Principles and Guidelines
- Publications
- Training
- Meetings and Workshops
- Expanded Guidelines
- Application Instructions

Two of the cornerstones of science advancement are rigor in designing and performing scientific research and the ability to reproduce biomedical research findings. The application of rigor ensures robust and unbiased experimental design, methodology, analysis, interpretation, and reporting of results. When a result can be reproduced by multiple scientists, it validates the original results and readiness to progress to the next phase of research. This is especially important for clinical trials in humans, which are built on studies that have demonstrated a particular effect or outcome.



Johns Hopkins University students in a laboratory. Johns Hopkins University

In recent years, however, there has been a growing awareness of the need for rigorously designed published preclinical studies, to ensure that such studies can be reproduced. This webpage provides information about the efforts underway by NIH to enhance rigor and reproducibility in scientific research.

Email Updates

Sign up to receive email updates about rigor and reproducibility.

[Sign up for updates](#)

Related Links

[Letter from Dr. Stephen I. Katz: An Update on the NIH Initiative to Enhance Research Rigor and Reproducibility](#)

Contact Us

Please send email to NIHReprodEfforts@od.nih.gov.

<http://www.nih.gov/science/reproducibility>

Raise Community Awareness

- Workshop in Summer 2014 with PhRMA to identify areas of common interest with industry
- Workshop in Summer 2014 with Journal Editors to identify common opportunity areas
- **Over 135 journals** endorsed the principles, which were broadly shared in November 2014 through editorials and other notifications

JCB

CellPress

nature

jbc THE JOURNAL OF
BIOLOGICAL CHEMISTRY

 PLOS | ONE

Science

Circulation

The Journal of Neuroscience

Raise Community Awareness

Efforts by Other Organizations: Recent Example



AMERICAN STATISTICAL ASSOCIATION
Promoting the Practice and Profession of Statistics®

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AMERICAN STATISTICAL ASSOCIATION RELEASES STATEMENT ON STATISTICAL SIGNIFICANCE AND *P*-VALUES

*Provides Principles to Improve the Conduct and Interpretation of Quantitative
Science*

“We teach it because it’s what we do; we do it because it’s what we teach.”

<http://amstat.tandfonline.com/doi/abs/10.1080/00031305.2016.1154108#.Vt2XIOaE2MN>]. The ASA

“The *p*-value was never intended to be a substitute for scientific reasoning”

chosen techniques, properly conducted analyses, and correct interpretation.

Principles for Addressing Underlying Issues

- Raise community awareness
- **Enhance formal training**
- Protect the quality of funded and published research by adoption of more systematic review processes
- Share information/data
- Increase stability for investigators

Enhance Formal Training

- NINDS, IRP, and Office of the Director (OD) developed training modules in experimental design, which are being used within the IRP and are available publicly
- NIGMS (with 9 other ICs) is supporting the development of training modules to enhance reproducibility
 - Funded 6 awards, supported by 8 ICs
 - Planning to re-issue the RFA
- IRP workshops on data interpretation considerations for various experimental techniques – “potentials and pitfalls”



Principles for Addressing Underlying Issues

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Application and Review Processes

- The NIH Office of Extramural Research (OER) **clarified and revised application instructions and review criteria** to enhance reproducibility of research findings
- Enhancing reproducibility through rigor and transparency
 - Scientific premise of proposed research
 - Rigorous experimental design
 - Consideration of sex and other relevant biological variables
 - Authentication of key biological and/or chemical resources
- Considering sex as a biological variable in NIH-funded research
- Applies to application submitted **Jan. 25, 2016** and beyond

<http://grants.nih.gov/reproducibility>

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Share Information/Data

PubMed
share

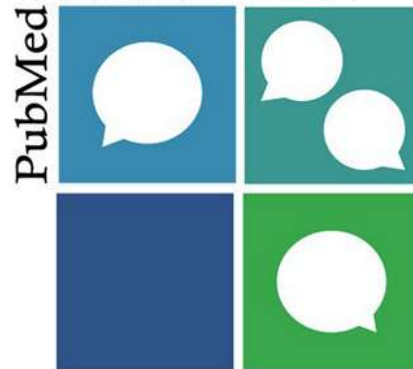
ers to
Med

NIH DIRECTOR'S BLOG

PubMed Commons: Catalyzing Scientist-To-Scientist Interactions

Posted on August 5, 2014 by [Dr. Francis Collins](#)

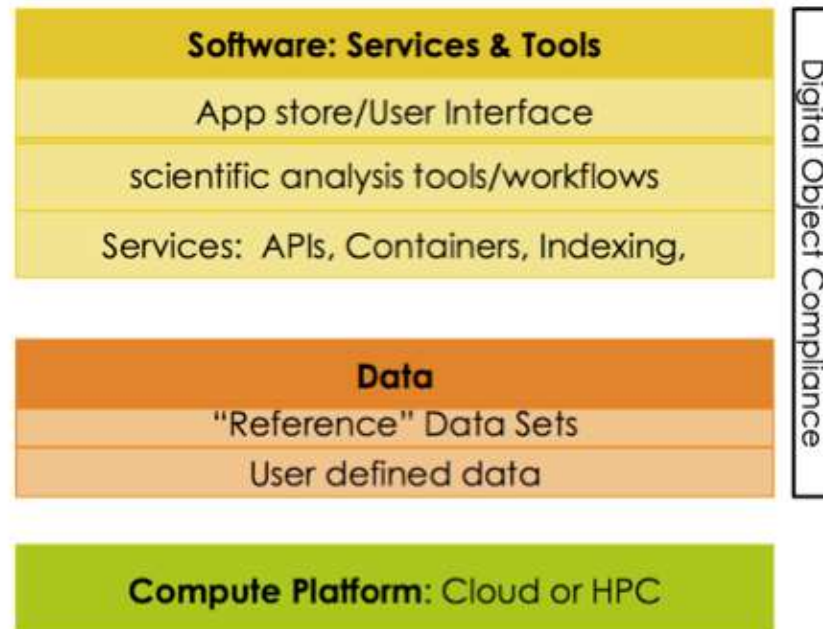
PubMed COMMONS BLOG



Today's scientists find it tough to keep up with all of the latest journal articles, innovative methods, and interesting projects of colleagues in their fields. That's understandable, because there are tens of thousands of journals, hundreds of conferences in major fields, dozens of emerging technologies, and huge geographic distances separating researchers who may share common interests. But science is increasingly a team sport—and it's important to provide scientists with as many avenues as possible through which to interact, including commenting on each other's work.

Share Information/Data

NIH Data Commons: Findable, Accessible, Interoperable and Reusable (FAIR)



- A **computing environment**, such as the cloud or High Performance Computing (HPC) resources, which supports access, utilization, and storage of digital objects
- Publicly available **datasets** that adhere to a *Commons* digital object compliance model
- **Software** services and tools to facilitate access to and use of data, both the data in the Commons or elsewhere
- A **digital object compliance model** that describes the properties of digital objects that enable them to be findable, accessible, interoperable, and reproducible (FAIR)

Share Information/Data

Efforts by Other Organizations: Recent Example



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Microbiology
Molecular Biology
Neuroscience
Paleontology

Pathology
Pharmacology
Physiology
Plant Biology
Scientific Communication
Synthetic Biology
Systems Biology
Zoology

View by Month

Share Information/Data

ILLUSTRATIONS BY PETE ELLIS



Team up with industry

Combining commercial and academic incentives and resources can improve science, argues **Aled Edwards**.

Share Information/Data

Efforts by Other Organizations: Recent Example

The screenshot shows the F1000Research website interface. At the top, there is a navigation bar with 'F1000Research / Channels' on the left and 'My Research | SIGN IN' on the right. Below this is a header for the channel 'Preclinical Reproducibility and Robustness' with a logo of two flasks. A secondary navigation bar includes 'Browse', 'How to Publish', 'About this Channel', and 'About F1000Research'. The main content area is titled 'Browse Channel' and features a search bar. On the right side, there is a prominent orange button labeled 'SUBMIT TO THIS CHANNEL'. Below the search bar, there is a section for 'Articles (4)' with a 'SHOW FILTERS' button. Two article entries are visible, both marked as 'AWAITING PEER REVIEW'. The first article is titled 'Effect of LXR/RXR agonism on brain and CSF Aβ40 levels in rats [version 1; referees: awaiting peer review]' by Songli Wang, Paul Wen, and Stephen Wood, published on 04 FEB 2018. The second article is 'Does inactivation of USP14 enhance degradation of proteasomal substrates that are associated with neurodegenerative diseases? [version 1; referees: awaiting peer review]' by Daniel Ortuno, Holly J. Carlisle, and Silke Miller, also published on 04 FEB 2018. On the right side of the page, there is an 'ABOUT THIS CHANNEL' section with a 'TRACK' button. The text describes the channel as a platform for open and transparent publication of confirmatory and non-confirmatory studies in biomedical research. Below this, there is a 'MORE ABOUT THIS CHANNEL' link and a 'Guest Editors' section featuring two profiles: Bruce Alberts (University of California, San Francisco, USA) and Alexander Kamb (Amgen Inc., USA).

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Investigator Stability



NIH DIRECTOR'S BLOG

Formula for Innovation: People + Ideas + Time

Posted on July 17, 2014 by [Dr. Sally Rockey](#) and [Dr. Francis Collins](#)

In these times of tight budgets and rapidly evolving science, we must consider new ways to invest biomedical research dollars to achieve maximum impact—to turn scientific discoveries into better health as swiftly as possible. We do this by thinking strategically about the areas of research that we support, as well as the process by which we fund that research.

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 the reproducibility of biomedical research is failing and is in need of restructuring^{1,2}. As leaders of the US National Institutes of Health (NIH), we share this concern and here explore some of the significant interventions that we are planning.

Science has long been regarded as 'self-correcting', given that it is founded on the replication of prior work. Over the long term, that principle remains true. In the

shorter term, however, the imbalances that once existed have been hobbling the ability of today's scientists to replicate others' findings.

Let's be clear: we have no evidence that reproducibility is about to improve. In 2011, the Office of the US Department of Health and Human Services pursued a strategy. Even if this represents the actual problem

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

Role for Individual Scientists

What you can do:

- Stimulate discussion amongst societies/organizations
- Increase transparency
- Promote training in experimental design
- Encourage data and material sharing
- Consider publication of refutations



NIH...

Lawrence.Tabak@nih.gov

Turning Discovery Into Health

