Why do we need scientific rigor and transparency in research?

NIH: The goal of this initiative is to enhance reproducibility of research through rigor and transparency.

Scientific Premise: Susan Pinney, PhD
Scientific Rigor: Kelly Brunst, PhD
Relevant Biological Variables: Divaker Choubey, PhD
Resource Authentication: Katie Burns, PhD

CEG
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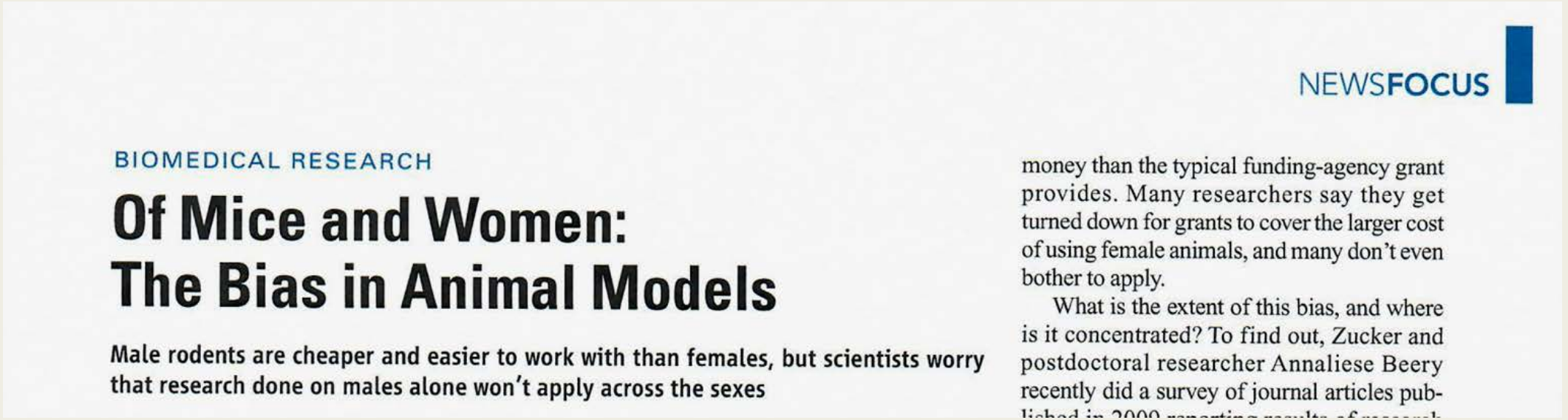
Scientific Rigor: A Practical Approach. Perspectives from Human & Basic Studies, 2018 Nov 15

Rigor and Transparency in Research

*To support the **highest quality science, public accountability, and social responsibility in the conduct of science**, NIH's Rigor and Transparency efforts are intended to clarify expectations and highlight attention to four areas that may need more explicit attention by applicants (and reviewers):*

- ***Scientific premise (significance)***
- ***Scientific rigor (approach)***
- ***Consideration of relevant biological variables, such as sex (approach)***
- ***Authentication of key biological and/or chemical resources (attachment)***



NEWSFOCUS

BIOMEDICAL RESEARCH

Of Mice and Women: The Bias in Animal Models

Male rodents are cheaper and easier to work with than females, but scientists worry that research done on males alone won't apply across the sexes

money than the typical funding-agency grant provides. Many researchers say they get turned down for grants to cover the larger cost of using female animals, and many don't even bother to apply.

What is the extent of this bias, and where is it concentrated? To find out, Zucker and postdoctoral researcher Annaliese Beery recently did a survey of journal articles pub-

Male rodents are cheaper and easier to work with than females, but scientists worry that research done on males won't apply across the sexes

[Science 327: 1571 (2010)]

ASTRO-H project manager Tadayuki Takahashi, an astrophysicist at the University of Tokyo and ASTRO-H's project manager, pushed his collaboration to work without borders. "Usually, international coalitions have clearly defined interfaces," with different laboratories providing isolated components of a spacecraft and its payload, says Takahashi. But ASTRO-H researchers regularly visited each other's labs, sometimes for months at a time.

Kelley says that Takahashi forged a very

IN FOCUS NEWS

REPLICATION

Biotech giant posts negative results

Amgen papers seed channel for discussing reproducibility.

In 2012, Amgen researchers declared that they had been unable to reproduce the findings in 47 of 53 "landmark" cancer papers (Nature 483: 531, 2012).

Nature 530: 141 (2016)

Scientific Premise

Scientific Premise refers to the quality and strength of the prior research used as the basis for the proposed research question or project; this is distinct from the hypothesis or justification.

- The applicant should discuss the strengths and weaknesses of the prior research used to support the application and describe how the proposed research will address weaknesses or gaps identified by the applicant.
- For example, a discussion of scientific premise might include attention to the rigor of previous experimental designs, either conducted by the applicant or reported in the literature.

Scientific Premise

GOAL: To ensure that the underlying scientific foundation of the project—concepts, previous work, and data (when relevant)—is sound.

- Provide sufficient justification for the proposed work
- Cite appropriate work and/or preliminary data
- Appropriately identify strengths and weaknesses in prior work (approaches) in the field
- Propose to fill a significant gap in the field
  (or explain why this is not possible?)

Scientific Premise - Reviewers

Reviewers will evaluate scientific premise as part of the Significance criterion for research grant applications or the Research Plan criterion for mentored career development award applications.

- Consider whether the applicant has discussed the strengths and weaknesses of the foundational data.
- A weak scientific premise, or the failure to address scientific premise adequately, may affect criterion and overall impact scores.
- The page limit is not an acceptable excuse for an applicant to not address scientific premise.

Scientific Premise – Example

While EMS lesions are a mixture of uterine and immune cells, it is not known how uterine tissue from retrograde flow survives, proliferates, and establishes lesions in the peritoneal cavity. Through this aim, we will elucidate uterine cell type and signaling molecules that contribute to the first phase of EMS development, uterine cell survival. Surgeons find that retrograde flow of menstrual tissue is normal in women[2]; therefore, a premise to our proposal is uterine cells must survive in the peritoneal cavity to form endometriotic lesions.

In both cascades, IL6 binding activates kinase-dependent signaling (STAT, MAPK, JAK), which ultimately regulates target genes (e.g. cyclooxygenase-2 (COX2), IL6 itself, IL6Rα, IL17, matrix metallopeptidases (MMPs), VEGF, basic fibroblast growth factor (bFGF), nuclear factor kappa of B cells (NFkB), and apoptotic genes[57,58]. Importantly and key to our premise, women with EMS have misregulation of many of these same factors (e.g., pSTAT3, MMPs, VEGF, COX2, IL6)44-56 and sIL6R is increased in PF compared to healthy women[72,73].

K Burns

Scientific Premise – Examples

A. Our scientific premise is that aberrant DNA methylation at specific loci can be inherited as well as acquired by lifestyle and environmental factors, and, in combination with genetic risk variants, will have greater prognostic value than either genetic or epigenetic risk factors alone.

B. Our scientific premise is that exposure to environmental chemicals with reported endocrine disrupting properties will affect peri-thelarcheal hormone serum concentrations, which then may consequently affect age at pubertal milestones and other pubertal maturation parameters.

C. Our scientific premise is that markers of early pubertal development are associated with the presence of severe headaches and migraine (SHMH) during later adolescence in girls.

S Pinney

Scientific Premise – Examples

A. Our scientific premise is that ultrafine particle exposure will result in a unique metabolomic profile in plasma that can be used to predict asthma phenotypes among adolescents.

B. Our scientific premise is that psychosocial stress-induced mitochondrial dysfunction in the placenta will have downstream effects on neuropsychological functioning in early childhood, particularly among ethnic-minority populations so heavily burdened by chronic adversity and trauma.

K Brunst

Scientific Rigor

GOAL: To ensure a strict application of scientific method (approach) that supports robust and unbiased design, analysis, interpretation, and reporting of results, and sufficient information (in publiations) for the study to be assessed and reproduced.

- *determine group size(s)*
- *analyze anticipated results*
- *reduce bias*
- *ensure independent and blinded measurements*
- *improve precision and reduce variability*
- *include or exclude research subjects*
- *manage missing data*

Scientific Rigor (Design)

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

Emphasize how the experimental design and methods proposed will achieve robust and unbiased results.

Scientific Rigor – Examples

- Experimental design and methodology
  - A. *Environmental biomarkers conducted at internationally recognized laboratories for high quality analytics and quality assurance requirements*
    - CDC Environmental Laboratory
      - *Clinical Laboratory Improvement Amendments certification which allows results to be returned to participants*
  - B. *Administration of 10% of all behavioral tasks are observed confidentially by a PhD psychologist, via a 1-way mirror to monitor tester performance and provide feedback*
  - C. *Randomization and blinding of animal groupings/data collection*

Scientific Rigor – Examples

- Unbiased analyses, interpretation, and reporting of results

A. *Pre-specified analytic plans with mandated reporting of null results*

B. *Required analysis verification by a second data analyst and code sharing prior to publication*

C. *Propose sharing data in conjunction with publications to enhance the replicability of our findings*

Relevant Biological Variables

GOAL: To ensure that the research accounts for sex and other relevant biological variables in developing research questions and study designs. The ways in which sex and other biological variables need to be accounted for will differ across research questions and fields of study.

Specific considerations for applicants:

- *Apply broadly to all biological variables relevant to the research such as sex, age, source, weight, or genetic strain*
- *Consider biological variables, such as sex, that are relevant to the experimental design*
- *Relevant biological variables are controlled or factored into the study design appropriately*

Sex as a Biological Variable

Consideration of sex, include under the umbrella of “Relevant Biological Variables” for all studies involving human subjects or vertebrate animals.

- If little is known about sex differences, the application should include both sexes in proposed research plan
- If sex differences are known not to exist, a strong justification should be provided if the application proposes to study one sex
- If sex differences are known to exist, experiments should be designed with appropriate group sizes to detect sex differences

Biological Variables for Studies Involving Animals

- Genetic background (e.g., C57BL/6 *versus* Balb/c)

- Vendor source/supplier

- Age of the animals

- Housing conditions (room temperature, light/dark cycle)

- Diet (low/high fat)

Biological Variables for Studies Involving Humans and Samples

- Race
- Age
- Body mass index (BMI)
- Socioeconomic status
- Underlying health conditions

Biological Variables – Examples

A. We also will control for age, sex and smoking history in the analysis.

B. Each methylation trait will be regressed against sex, age, batches and PCA scores based on methylation profiles.

S Pinney

Biological Variables – Examples

A. Certain neuropsychological conditions including ADHD, anxiety, and depression present a sex bias
B. Sex differences in mitochondrial biogenesis in response to environmental stimuli are known
C. Sex-specific effects of trauma and particulate air pollution on placental mtDNAcn are documented
D. Sex differences in mitochondria biogenesis, morphology, and respiratory function have been noted
E. Sex differences in trans-placental signaling may also influence neuro-programming

K Brunst

Plan for Resource Authentication

GOAL: To ensure processes are in place to identify and regularly validate key resources used in their research and avoid unreliable research as a result of misidentified or contaminated resources.

- Authenticate key biological and/or chemical resources used in research to ensure that the resources are genuine.

Authentication

Key biological and/or chemical resources:

- cell lines, specialty chemicals, antibodies, biologics.

What is needed:

- Brief description of methods to ensure the identity and validity of key biological and/or chemical resources used in the proposed studies.
- These resources may or may not be generated with NIH funds and may differ from laboratory to laboratory or over time; may have qualities and/or qualifications that could influence the research data; are integral to the proposed research.

Authentication

The authentication plan should state in one page or less:

- how you will authenticate key resources,
- the frequency you will authenticate

- Note: Do not include authentication data in your plan.

Authentication- Example

Epidemiology study proposing gene expression analyses (R01)

All reagents used in sample processing in preparation for gene expression array experiments are of standard analytical grade and are purchased from reliable commercial sources such as Qiagen and ThermoFisher and are readily available to the scientific community. Standard biological reagents have been authenticated prior to receipt by the vendor as detailed by the certificate of analysis included with each product. We also take additional measures in data quality control for RNA samples given that this proposal is dependent on high quality expression data. The concentration and purity of the isolated RNA will be determined by spectrophotometry using a NanoDrop® ND-1000 and by electrophoretic separation. Expression of housekeeping/control genes will be measured by qPCR for each collected sample. In addition, several additional quality control steps are included to ensure PCR array reproducibility, real-time efficiency, and the lack of genomic DNA contamination.

K. Brunst

Authentication- Example

Epidemiology study proposing metabolomics (R21)

All reagents used in sample processing in preparation for NMR and MS assay are of standard analytical grade and are purchased from reliable commercial sources such as Fisher Scientific, Chenomx (www.chenomx.com), and Cambridge (https://www.isotope.com/) and are readily available to the scientific community. Authentication of key biological components for NMR analyses will occur by both spiking samples with standard known compounds and by the collection of two-dimensional proton-proton and proton-carbon NMR experiments, which provide structural information that aids in the assignments of important metabolites. Standard biological reagents purchased for MS analyses have been authenticated prior to receipt by the vendor as detailed by the certificate of analysis included with each product. However, we will perform our own authentication by conducting a full scan analysis using MS and assessing molecular weight fragmentation and retention time of each compound.

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Authentication- Example

All products will be purchased from established scientific vendors and authentication will be requested with each product. All products will be re-validated intermittently within the grant period and with each new order to ensure reproducibility and consistency across the experiments.

Products used in this project include the following with verification methods:

1. rhVEGF165—will be purchased from R&D Systems and the activity will be verified by examining the increase in neutrophils in the mouse peritoneal cavity. Neutrophils will be stained with Ly6G and will be compared against mice not receiving rhVEGF165 treatment. References: Byrne, A.M. et al. (2005) J Cell Mol Med 9:777. Pan, Q. et al. (2007) J Biol Chem 282:24049.

2. sgp130FC-protein—will be gifted to us by Dr. Rose-John, Institute of Biochemistry, University of Kiel, Germany. Activity of the protein will be verified by ELISA or Western blot to confirm the effects of the compound in our model. References: Garbers, C. et al. (2011) J. Biol. Chem. 286(50):42959.

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K. Burns

Authentication- Example

3. Clodrinate Liposomes—will be purchased from an established vendor and the activity will be verified by cell differential staining of the peritoneal cavity or by flow cytometry for macrophages. References: Biewenga, J. et al. (1994) Cell Tissue Res.280. Weisser, S.B. et al. (2012) J. Vis. Exp. (66), e4105.

4. Antibodies—each antibody will be purchased from a known company such as eBiosciences, BioLegend, Tonbo, Abcam, R&D Systems, Sigma, etc. Each of the antibodies used in flow cytometry will be tested as single color controls to verify the validity of each single antibody to be used in a comprehensive immunophenotyping panel. Antibodies used for immunohistochemistry will be validated on positive and negative control samples and will be tested for specificity with controls (no primary, no secondary, and control IgG). ….

7. Mouse strains—all mouse strains, if needed for alternative approaches, will be genotyped for proper genetics before use. Mice expressing green fluorescent protein are homozygous and fluoresce under UV light. References: Dickinson ME. et al. (2016) Nature 537(7621): 508-514., Schaefer BC.

K. Burns

Rigor and Transparency of Research:

Item	Applies to which applications?	Where will I find it in the application?	Where do I include it in my critique?	Affect overall impact score?
Scientific Premise	All	Research Strategy (Significance)	Significance	Yes
Scientific Rigor	All	Research Strategy (Approach)	Approach	Yes
Consideration of Relevant Biological Variables, Such as Sex	Projects with vertebrate animals and/or human subjects	Research Strategy (Approach)	Approach	Yes
Authentication of Key Biological and/or Chemical Resources	Project involving key biological and/or chemical resources	New Attachment	Additional review considerations	No

Additional resources

- Rigor and Reproducibility in grant applications (OER site): http://grants.nih.gov/reproducibility/index.htm
- NIH presentation of background and goals of Rigor and Transparency (video) https://grants.nih.gov/reproducibility/module_1/presentation.html
- Reviewer Guidance on Rigor and Transparency: http://grants.nih.gov/grants/peer/guidelines_general/Reviewer_Guidance_on_Rigor_and_Transparency.pdf
- Consideration of Sex as a Biological Variable in NIH-funded Research http://orwh.od.nih.gov/sexinscience/overview/pdf/NOT-OD-15-102_Guidance.pdf
- Rigor and transparency do not apply to all applications. See List of Eligible Activity Codes: https://nih-extramural-intranet.od.nih.gov/d/sites/default/files/RigorActivityCodes-20151006.pdf. Also, certain Funding Opportunity Announcements are exempt from Rigor and Transparency, by request from the ICs.

- Questions about the NIH policy should be directed to reproducibility@nih.gov

Thank You
Questions?

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