# Rigor in the Research Approach

Blind them with science
How experiment tests hypothesis
Conceptualize what results will be, if right, wrong
Aims 1 and 2 about the same length

- Restate each Aim as on SA page
- Reiterate rationale for each Aim
- Describe how literature, pilot data support your approach
- State how your study will address the gap in knowledge
- Describe the overall strategy, methodology, and analyses
- Include how the data will be collected, analyzed, and interpreted. Consider design, sample size, power,
- Describe expected results, potential problems and alternative approaches

# Rigor and Reproducibility

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

- Strong experimental design
- Methodology
- Analysis
- Interpretation

For most applications, you need to address Rigor and Reproducibility by describing the experimental design and methods you propose and how they will achieve robust and unbiased results.

# Four areas of rigor and transparency:

1. Rigor of prior research
2. Rigorous experimental design robust and unbiased
3. Consideration of biological variables
4. Authentication of key biological resources

https://grants.nih.gov/policy/reproducibility/guidance.htm

…fellowship candidates will be expected to address, as applicable, any new research skills they plan to acquire in the areas of rigorous research design, experimental methods, quantitative approaches, and data analysis and interpretation.

…fellowship candidates will be expected to describe (a) the strengths and weaknesses in the rigor of the prior research that serves as the key support for the proposed project, (b) plans to address any weaknesses in the rigor of the prior research, (c) how the experimental objectives proposed will achieve robust and unbiased results, and (d) how relevant biological variables are factored into research designs and analyses.

•If applicable…include the Authentication of Key Biological and/or Chemical Resources attachment.

# What's the fuss about rigor?

In 2012, Amgen researchers made headlines when they declared that they had been unable to reproduce the findings in 47 of 53 'landmark' cancer papers[1]

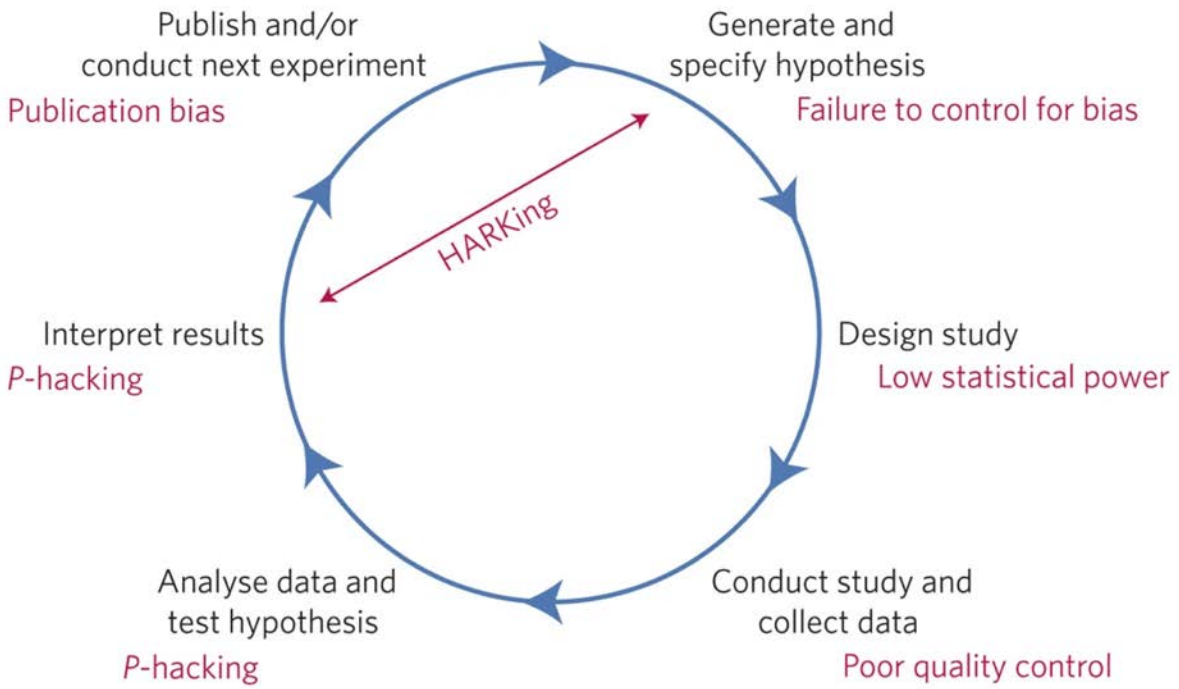
Common reasons for lack of reproducibility:

- Usually not scientific misconduct
- Report only positive, large results
- Incorrect or inappropriate statistical analysis of results
- Insufficient sample sizes
- Poor experimental design
- No blinding, randomization, replication, sample size, sex differences

Lack of reproducibility abetted by culture of science

- Competition to publish
- Negligent reporting of experimental conditions
- Insufficient description of materials and methods
- Bias towards publishing positive results
- Conflict of interest

From: A manifesto for reproducible science




An idealized version of the hypothetico-deductive model of the scientific method is shown. Various potential threats to this model exist (indicated in red), including lack of replication[5], hypothesizing after the results are known (HARKing)[7], poor study design, low statistical power[2], analytical flexibility[51], *P*-hacking[4], publication bias[3] and lack of data sharing[6]. Together these will serve to undermine the robustness of published research, and may also impact on the ability of science to self-correct.

# What happened next at Journals

In 2014 NIH “principles and guidelines”

Asked journals to modify editorial process
document statistical analyses
use best practices reagent verification
identify big data sources, data elements,
curate final analytic set, “data wrangling”

- Landis et al 2012 A call for transparent reporting to optimize the predictive value of preclinical research *Nature* **490**, pages187–191
- Ioannidis 2005 paper "Why Most Published Research Findings Are False".[54]
- Begley and Ionnidis 2015 “Reproducibility in science: improving the standard for basic and preclinical research” Circ Res 116: 116-26
- Munafo et al 2017 A manifesto for reproducible science. Nature Human Behavior

Nature Publishing Group, BioMed Central AAAS 2013

- Abolish restrictions on the length of methods sections
- Sample size larger, misinterpretation of p values
- Characterize key reagents, cell lines and antibodies
- Checklist to facilitate verification by editors and reviewers
- Editors assisted by statisticians in evaluating studies \
- Key findings require validation by third party

Funding agencies

- Scientific premise of application-key pubs on which application is based
- NIH updated language, training modules

# Some journals now use evidence checklists …

## Han et al 2017

Searched two highly cited life science journals, one that requires a checklist at submission (*Nature*) and one that does not (*Cell*), to identify *in vivo* animal studies.

After screening 943 articles, a total of 80 articles were identified in 2013 (pre-checklist) and 2015 (post-checklist), and evaluated reporting methodological and analytical information.

Compared the quality of reporting preclinical animal studies between the two journals, accounting for differences between journals and changes over time in reporting.

We find that reporting of randomization, blinding, and sample-size estimation significantly improved when comparing *Nature* to *Cell* from 2013 to 2015, likely due to implementation of a checklist…

And what happened next at NIH

NIH Created Training Modules
1. Lack of transparency
2. Blinding and Randomization
3. Biological and technical replicates
4. Sample size, outliers and exclusion criteria

https://grants.nih.gov/policy/reproducibility/training.htm

Rigor and reproducibility
Should be woven throughout your research plan

# Describe rigor of prior research & your study

**Research Grants and Mentored Career Development Awards**
**Beginning with applications due on January 25, 2019** the application instructions and review criteria will be clarified to replace the term “scientific premise” with the term "**rigor of the prior research**". Applicants will also be instructed to describe plans to **address any weaknesses** in the rigor of prior research within the Research Strategy.

**Individual fellowship applications** will be required to summarize in the research strategy section **plans to ensure rigorous, well-controlled experiments that consider all relevant biological variables, use authenticated biological and chemical resources, and apply appropriate statistical tests for data analyses**. In addition more detailed description of instruction in rigorous experimental design to ensure reproducibility will be required in the section on Institutional Environment and Commitment to Training.

# Biostatistics are just the beginning

**Statistical analysis:** All *in vivo* experiments comparing the course of EAE will be performed with a minimum of 15 mice per genotype or treatment group with replication, and all *in vitro* experiments will be performed at least three times to ensure reproducibility of the findings. These experimental group sizes are based on power calculations and feasibility. Statistical analyses will be done in coordination with biostatistics resources and experts available at our institution. Pooled data will be expressed as mean±SEM for *in vivo* and mean±SD for *in vitro* experiments, and longitudinal measures assessed by repeated-measures ANOVA. When significant differences are observed ($p<0.05$) post-hoc pair-wise least-squared difference tests will be performed with Bonferroni corrections. When data are not normally distributed analysis will be performed after transformation.

F31 NINDS Wrights

# Some instruction from biostatistician

CTSI-CN SPARC request portal

| 4 AREAS OF FOCUS | WHAT DOES IT MEAN? | WHERE SHOULD IT BE INCLUDED IN THE APPLICATION? |
|---|---|---|
| **Rigor of the Prior Research** | A careful assessment of the **rigor of the prior research** that serves as the key support for a proposed project will help applicants identify any weaknesses or gaps in the line of research.<br><br>Describe the strengths and weaknesses in the rigor of the prior research (both published and unpublished) that serves as the key support for the proposed project.<br><br>Describe plans to address weaknesses in the rigor of the prior research that serves as the key support for the proposed project<br>*See related FAQs, blog post | **Research Strategy**<br>➢ Significance<br><br>➢ Approach |
| **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.<br><br>Emphasize how the experimental design and methods proposed will achieve robust and unbiased results.<br>*See related FAQs, blog post, examples from pilots | **Research Strategy**<br>➢ Approach |
| **Biological Variables** | **Biological variables**, such as sex, age, weight, and underlying health conditions, are often critical factors affecting health or disease. In particular, sex is a biological variable that is frequently ignored in animal study designs and analyses, leading to an incomplete understanding of potential sex-based differences in basic biological function, disease processes and treatment response.<br><br>Explain how relevant biological variables, such as the ones noted above, are 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.<br>*See related FAQs, blog posts, article | **Research Strategy**<br>➢ Approach |
| **Authentication** | **Key biological and/or chemical resources** include, but are not limited to, cell lines, specialty chemicals, antibodies and other biologics.<br><br>Briefly describe 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 have been generated with NIH funds and:<br>• may differ from laboratory to laboratory or over time;<br>• may have qualities and/or qualifications that could influence the research data;<br>• are integral to the proposed research.<br>The authentication plan should state in one page or less how you will authenticate key resources, including the frequency, as needed for your research. Note: Do not include authentication data in your plan.<br>*See related FAQs, blog post, examples | **Other Research Plan Section**<br>➢ Include as an attachment<br>➢ Do not include in the Research Strategy. |

# NIH ENHANCING REPRODUCIBILITY GUIDELINES

what you need to know

## WHAT ARE THE FOUR ELEMENTS OF RIGOR?

1. RIGOR OF THE PRIOR RESEARCH
2. RIGOR OF THE PROPOSED RESEARCH
3. BIOLOGICAL VARIABLES
4. AUTHENTICATION

Send inquiries to reproducibility@nih.gov

See also NIH Notice NOT-OD-18-228
https://grants.nih.gov/grants/guide/notice-files/NOT-OD-18-228.html

## WHERE IN THE APPLICATION?

### 1 RESEARCH STRATEGY

The research strategy is where you discuss the significance, innovation, and approach of your research plan. Let's look at an R01, for example:


The **research strategy** guidelines require that you:

- Describe the strengths and weaknesses in the rigor of the prior research that serves as key support.
- Describe plans to address weaknesses in the rigor of the prior research.
- Describe how your experimental design and methods will achieve robust and unbiased results.
- Explain how relevant biological variables, such as sex, are factored into research designs and analyses.

### 2 ATTACHMENT FOR AUTHENTICATION OF KEY BIOLOGICAL AND/OR CHEMICAL RESOURCES

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

These include, but are not limited to:

CELL LINES
ANTIBODIES
SPECIALTY CHEMICALS
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.

- **DO NOT** put experimental methods or preliminary data in this section
- **DO** focus on authentication and validation of key resources

### 3 REVIEW GUIDELINES

Here are the additional criteria the reviewers will be asked to use:

- Is the **prior research** that serves as the key support for the proposed project **rigorous**?
- Have the investigators included plans to **address weaknesses in the rigor of prior** research that serves as the key support for the proposed project?
- Have the investigators presented **strategies to ensure a robust and unbiased approach**, as appropriate for the work proposed?
- Have the investigators presented adequate plans to address **relevant biological variables, such as sex,** for studies in vertebrate animals or human subjects?

Reviewers will also be asked to comment on that new attachment (see **Update 2**)!

Some questions:

Replication

Within an individual experiment, what do you think is the best approach to determine the appropriate number of replicates?

Do you think it is common to report data from a single experiment (technical replicates) to generate an “exciting” finding? How often is this type of practice viewed as a way to expedite the research process?

- Since this is a grant application with preliminary results, is it acceptable to include results in such a manner?
- Do you think papers or grant applications should delineate the use of biological vs. technical replicates in the figure legends (or elsewhere in the document)?

Some questions:

Blinding, Bias?

- Can you think of a particular instance in which blinding and randomization could have a dramatic impact on your results?
- Have you ever blinded and/or randomized samples in your own experiments?
- How realistic is the feasibility of blinding (expertise in the lab, contact with potential blinders)?

Have you ever felt pressure, either from your PI or reviews of a submitted paper, to obtain a specific result? *For example, reviews state that the paper will be accepted for publication if the authors can demonstrate a particular result, which will increase the paper's significance.*

Some Questions:

Sample size

- Have you ever tried to replicate someone's experimental approach and discovered that information was missing in their lab notebook? decrypt their handwriting/abbreviations?
- Do you maintain a thorough laboratory record? If so, what methods do you follow to ensure that your lab notebook is comprehensive?
- Do you think an electronic lab notebook would have helped identify the issue(s) faster? What characteristics would the electronic lab notebook need to have?

Statistical Methods and Issues

- Have you ever had data that was "close" to significance? If so, what did you do? How did you interpret these results?

Work rigor into the research strategy

- premise of previous results
- study design, controls, variables
- controls and reproducibility plans
- authentication issues
- Transparency/data dissemination

Consider a standard approach to determining the appropriate sample size and setting criteria for outliers

Consider how you will determine the numbers that go into your power analysis

Consult with biostatistician ***before*** you do the experiment!

# Include rigor in career development plan

Applied biostatistics for basic research
Turorials on data management, mining,  programming
Lab meetings to discuss design & reproducibility
Consultation with biostatistician-SPARC

Study Design, Statistical Analyses and Quantitative Literacy.
good study design and appropriate use of quantitative methods

Good research practices for framing a research question
Illustrate the use of FINER, PICOT and SMART criteria,

ARRIVE guidelines for randomized animal studies,
STROBE guidelines for observational studies
Statistical inference and goodness of fit

Tailored to your project