

Animal Issues in Research Reproducibility

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Why Should We Care?


- **Increased administrative burden**
Cost
- **Diminished confidence in science by public**
- **Ammunition for the animal rights organizations**
 - **White Coat Waste**

Benefits of Animal Research

- Banting and Best—studies with dogs to understand role of insulin in diabetes
- Florey and Chain— penicillin in mice
- Citrate in blood as an anticoagulant in dogs
- Organ transplants using dogs, calves and pigs
- Vaccine development—animal studies led to the first polio vaccine and have been instrumental in subsequent vaccine research

More Recent Therapies and Animal Research

- Restasis (cyclosporine) was first used in dogs (Kaswan, 1989).
- Viagra was developed from animal studies on the nitric oxide system.
- Flu vaccines are studied in ferrets and chickens before moving to human use.
- Pembrolizumab (KEYTRUDA®)—immunotherapy used to treat President Jimmy Carter was developed using animal studies.

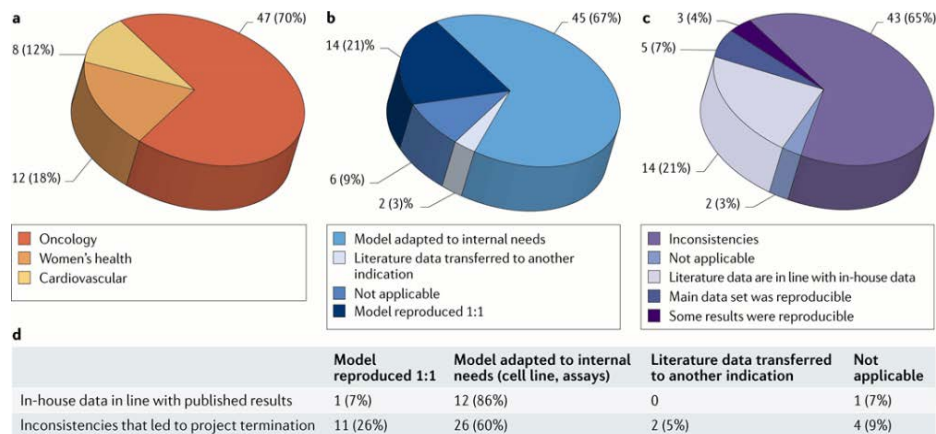


Animal studies have been, and continue to be, critical to biomedical research and to practically every advance we have made in modern medicine.

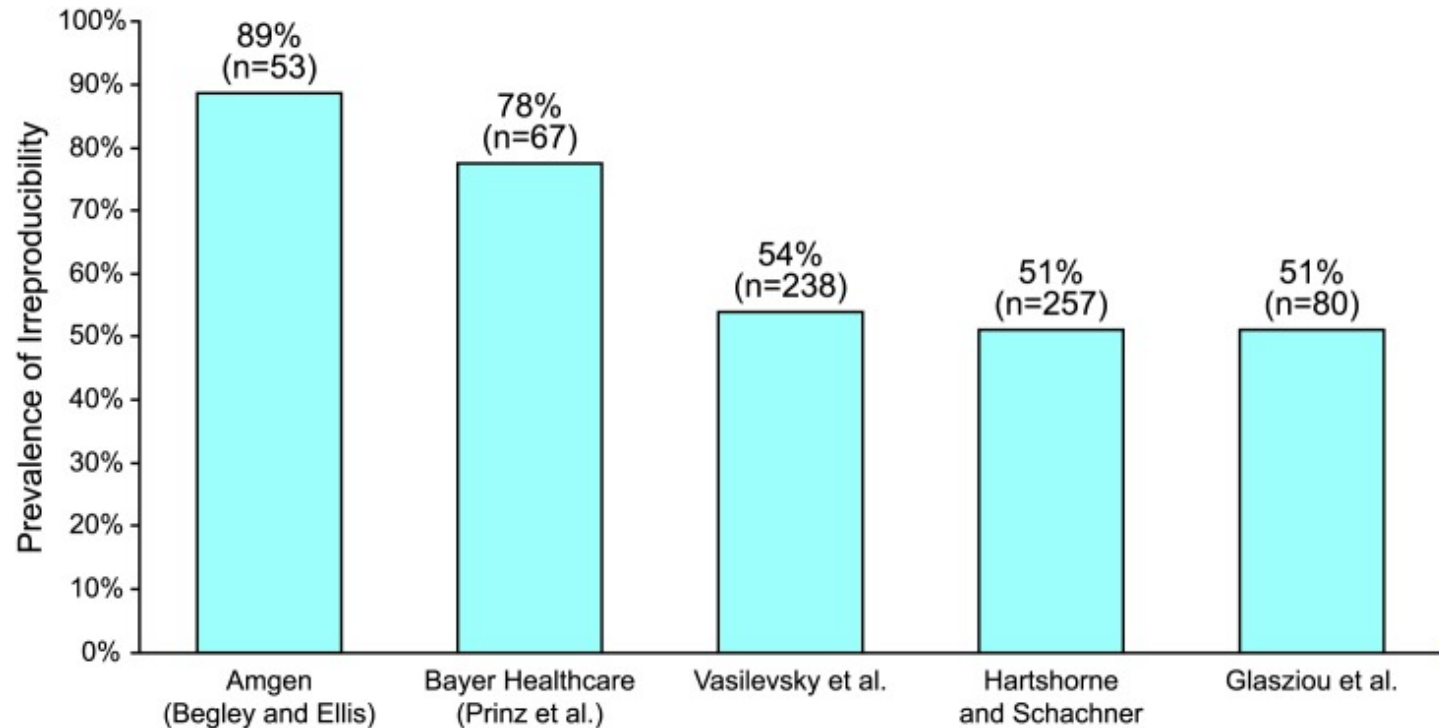
Increased Concern with Reproducibility

- Reproducibility as an issue for science has been recognized for some time (Ioannidis, 2005)
- Amgen (Begley & Ellis, 2012) and Bayer (Printz et al, 2011) were unable to replicate significant percentages of published studies

Begley & Ellis
-only 6 of 53 papers
were findings
confirmed

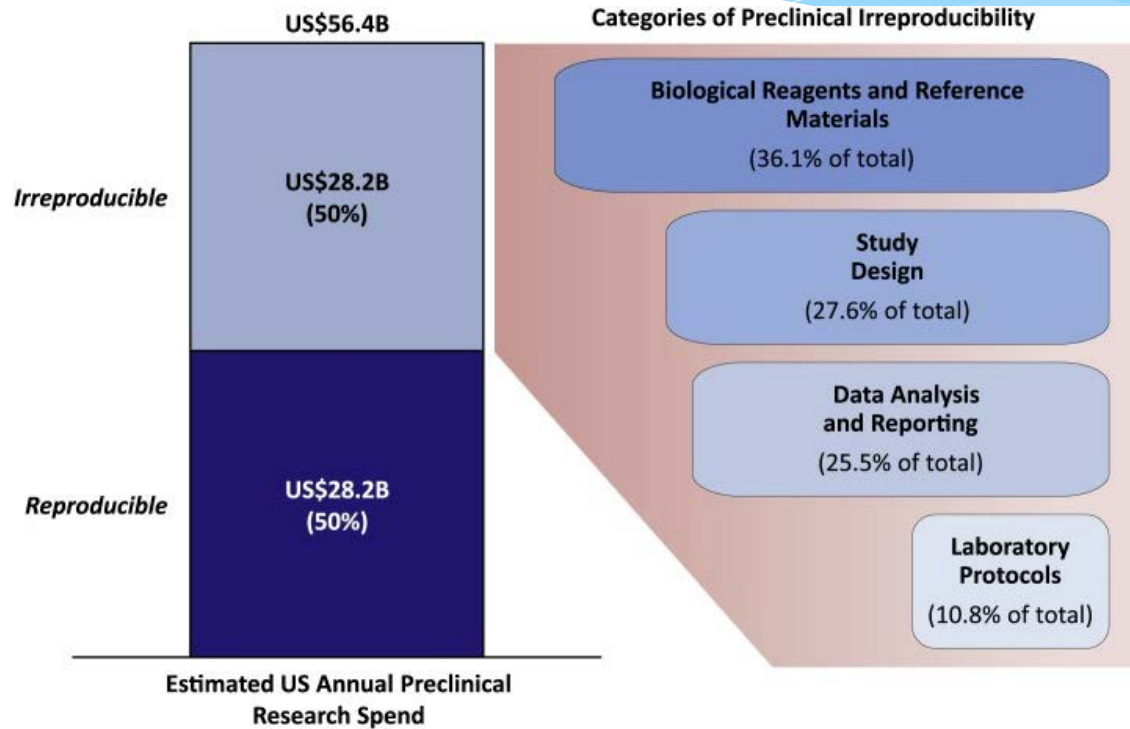


% of Studies Reported to be Irreproducible



Freedman LP, Cockburn IM, Simcoe TS (2015) The Economics of Reproducibility in Preclinical Research. *PLoS Biol* 13(6): e1002165. doi:10.1371/journal.pbio.1002165

Estimated Cost to the Preclinical Research Enterprise



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NIH Review Process Revised

Four areas deemed important for enhancing rigor and transparency

- 1) The scientific premise of the proposed research
- 2) Rigorous experimental design for robust and unbiased results
- 3) Consideration of relevant biological variables
- 4) Authentication of key biological and/or chemical resources

This increases administrative burden.

There are now independent organizations working to replicate aspects of what are viewed as high impact papers. ***This costs money!***

Contributing Factors to Poor Reproducibility

- Experimental design and statistics
 - Experimental design is often poor.
 - Small sample size with low statistical power
 - Poor training in statistics
 - Design and statistical models not considered prior to conduct of experiment
 - Data mining
 - “Fishing” for significant p-values
 - Subjects not randomized properly
 - Subjective measurements are not blinded

Contributing factors (cont)

- Research environment
 - Pressure to publish
 - Publish in high impact factor journals
 - Publish only positive findings
 - Very few negative data are published
 - Hard-to-get grant dollars
 - Increases pressure to publish, especially positive data
 - Increases pressure to publish underpowered studies
 - Pressure to confirm an hypothesis (investigator bias)
 - Failure to blind subjective observations
 - This bias contributes to the pressure to publish positive results

Contributing factors (cont.)

- **Reagent quality and verification**
 - **Antibodies**
 - Rarely validated and cross-reactivity is a major concern
 - **Cell lines**
 - Conservative estimates are that 15-25% of cell lines are not the cell line the investigator thinks they are using.
- **Translation of animal work to humans**
 - Often look for face validity instead of verifying that the construct and etiology of the model is valid

Begley's Six Rules for Reproducibility

- 1) Were studies blinded?
- 2) Were all results shown?
- 3) Were experiments repeated?
- 4) Were positive and negative controls shown?
- 5) Were reagents validated?
- 6) Were the statistical tests appropriate?

Focus on Animal Research



Best Supporting Role in a Medical Drama.

Perhaps you didn't know that rats and mice are the foundation for all medical research and that they have played a vital role in virtually every major medical discovery in history. Learn more about the essential need for animal research.

FOUNDATION FOR BIOMEDICAL RESEARCH

www.fbresearch.org

Contributing Animal Factors

- **Is the model appropriate?**
- **Are the tests valid?**
 - Many behavioral tests are often misinterpreted
- **Age and weight of animal**
 - Vendor-supplied animals often have the DOB within the week
 - Weight at a particular age will vary among strains

Contributing Animal Factors (cont)

- **Thermoregulation**
 - **Temperature impacts research results**
 - Eg. Kokolus KM et al, PNAS 110; 20176, 2013 Housing at a higher temperature reduced tumor formation, growth rate and metastasis.
 - How many animals per cage?
 - Type of enrichment?
 - Type of food?
- **Vision**
 - * Albino vs pigmented?
 - * Where positioned on rack?
 - * Cage color?

Contributing Animal Factors (cont)

- **Olfactory cues**
 - * Other animals in room?
 - * Caretaker?
- **Microbiome**
 - * Some bacteria influence the development of the gut immune system
 - * Normal vs abnormal flora?
 - * Influenced by many factors
 - * Gnotobiotic vs specific pathogen free
 - * Gnotobiotic animals have a known flora
 - * Specific pathogen free animals are free of the pathogens one tests for

Contributing Animal Factors (cont)

- Housing density of animals
- **Enrichment**
 - How much?
 - Is it consistent?
- Type of food
- Humidity
- Shipping factors
- Experimenter

Role for the Veterinarians and Animal Care Staff


- * Veterinarians have knowledge of comparative physiology and medicine, as well as many animal models.
- * Animal care staff are front-line, day-to-day hands on with animals
 - Often first to note changes in animal behavior or to see signs of disease
- * Need to incorporate veterinarians and animal care staff into research team
 - Understand the goals of the research project
 - Can contribute to better husbandry practices

Suggestions for future

- Improved instruction in research design and statistics.
 - Begley's 6 Rules
- Instruction on proper record keeping.
- Publication of all data, including negative findings.
 - This will require a change in publication culture
- Better review by granting agencies and publications.
 - NIH is starting this. Consequences??

Suggestions for future with animal research

- Science-based husbandry practices
- Ensure investigators know the environment their animals live in
 - Provide the husbandry information to the investigators.
 - Could be in the form of a checklist so investigator can select relevant information
- Involve the veterinarian(s) and husbandry staff



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