Sex bias in preclinical research: why it exists and why change has been slow.

Natasha Karp
Quantitative Biology, Discovery Science, R&D, AstraZeneca, UK

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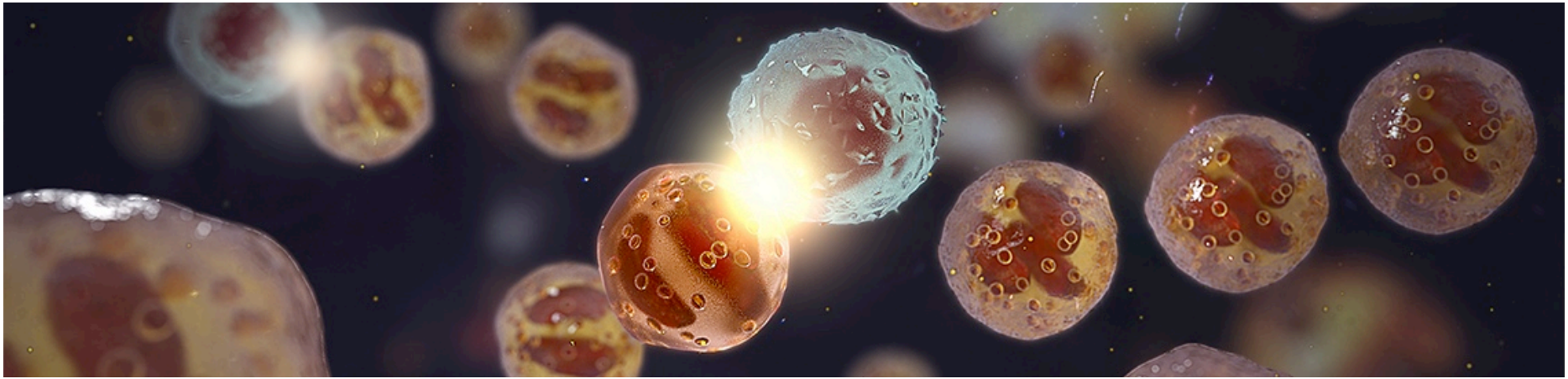


CALL CONSIDER SEX AS A BIOLOGICAL VARIABLE

The neglect of sex within *in-vivo* preclinical research

- **Reporting**:
  - Sex not specified – 22% did not specify

    Yoon et al 2014 Surgery
- **Experimental design**:
  - Study across 10 fields of biology = 80% ♂ rodents

    Beery and Zucker 2011 Neurosci Biobehav Rev
  - Sex bias has not changed 20 year period

    Mazure and Jones 2015 BMC Women's health
- **Analysis**:
  - When both sexes, only 33% analysed by sex

    Beery and Zucker 2011 Neurosci Biobehav Rev

What about *in-vitro* studies?

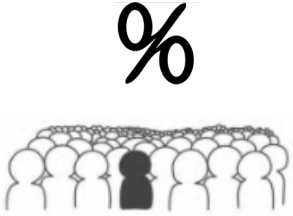
- **Reporting**:
  - 72-80% did not report sex Taylor 2011 Biol Sex Diff
  - 75% did not report the sex Shah 2014 AJP Cell Physiol
- **Experimental design**:
  - 80% only male Shah 2014 AJP Cell Physiol
  - 71% only males Yoon Surgery 2014
  - 69% male only Taylor 2011 Biol Sex Diff
- **Provision**:
  - Majority of cells are sold without defining the sex

Lee 2018 BMB Rep

What about ♀ prevalent disease?

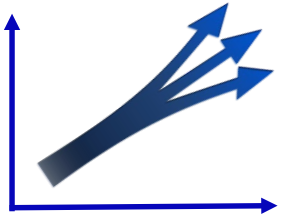
- Yoon *et al* 2014 Surgery
- Reviewed surgical biomedical research published 2011-2012
- For publications on ♀ prevalent diseases (n=45)
  - 44% did not report the sex studied
  - For those that reported the sex, only 12% studied ♀

Personalised medicine: sex

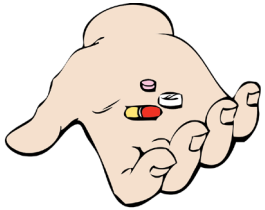


Prevalence

Symptoms



Progression



Side Effects

- 8/10 drugs withdrawn from the US market from 1997 to 2000 posed greater health risks for women than for men
- Adverse drug reactions were initially underappreciated in human females "including life-altering, disfiguring surgical complication, birth defects in babies and onset of chronic disease"

  US General Accounting Office (2001) Drug Safety: Most Drugs Withdrawn in Recent Years Had Greater Health Risks for Women (Government Publishing Office, Washington, DC).

- ADR Odds ratio: 1.596 (CI: 1.3-1.94, p <0.0001)

Sex as a biological variable

- Clayton and Collins 2014 **Nature**

  "NIH to balance sex in cell and animal studies"

Raised need to:

- Include females
- Analyses by sex
  - **SABV**: sex as a biological variable.
- Both cells and *in-vivo* studies
- **Importance**? NIH largest funders of biomedical research

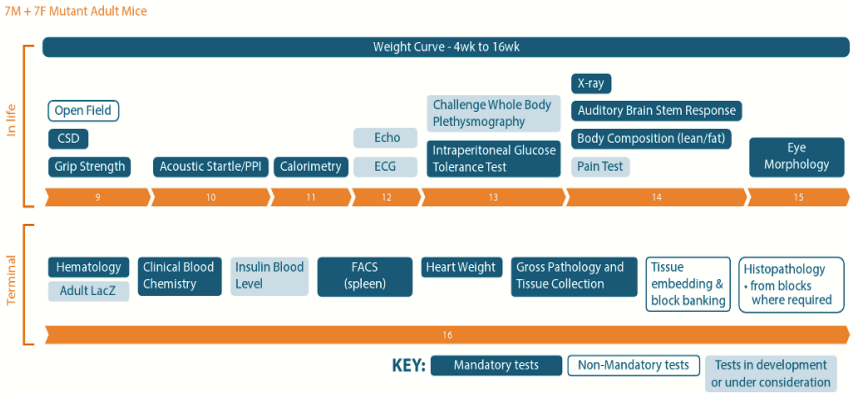
Moses et al 2015 JAMA

Controversy

- Field, R **Nature** 2014
  - “major step in the wrong direction” , “Waste of resources”
- Sandberg, K **Am J Physiol Regul Integr Comp Physiol** 2014
  - Unnecessary duplication and slow progress.
- Richardson **PNAS** 2015
  - question value of preclinical research in to sex differences
- Ritz **FASEB journal** 2013
  - Challenging – issues with confounders and sex isn’t binary.
- McCullough **Nature** 2014
  - “Sex must be evaluated in the context of other variables, such as age, experience, genetics and environment.”
- Johnson **PLoS ONE** 2014
  - **Knowledge gap**

ASSESSING THE PREVALENCE OF SEXUAL DIMORPHISM

Within preclinical research sex has been shown to matter



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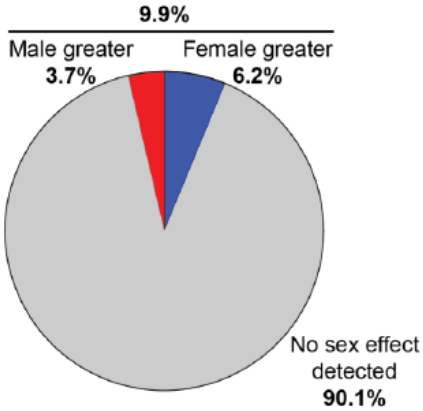
Prevalence of sexual dimorphism in mammalian phenotypic traits

Natasha A. Karp[1,2], Jeremy Mason[3], Arthur L. Beaudet[4], Yoav Benjamini[5], Lynette Bower[6], Robert E. Braun[7],

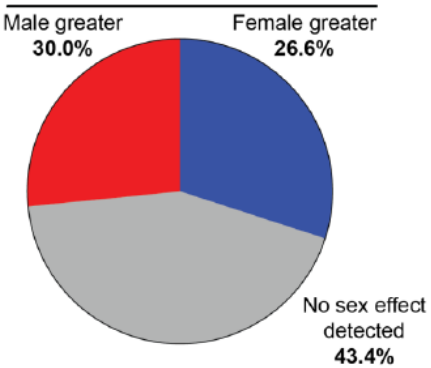
- 10 institutes
- 14,250 wildtype mice
- 40,192 mutant mice
- 2186 mutant lines
- up to 234 traits.

SABV?

In control data

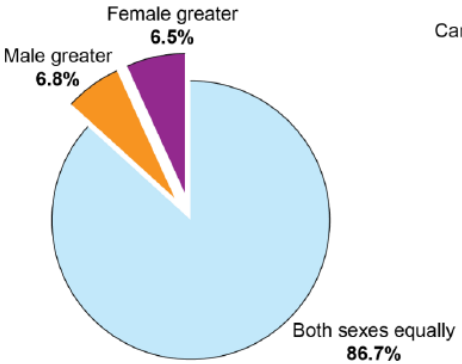


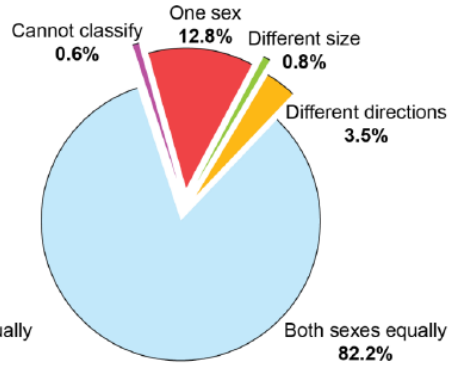
Categorical
N=545



Continuous
N=903

As a modifier of treatment effect?





Categorical

No ds = 266,952
No ds sig = 1,220

Continuous

No ds = 110, 586
No ds sig = 7 929

Karp et al 2017 Nature Communications

WHY DOES A SEX BIAS EXIST?

MISCONCEPTIONS

SKILL GAP

PRACTICAL CONCERNS

3R INTREPRETATION

Why? *In-vivo* - ethical framework interpretation

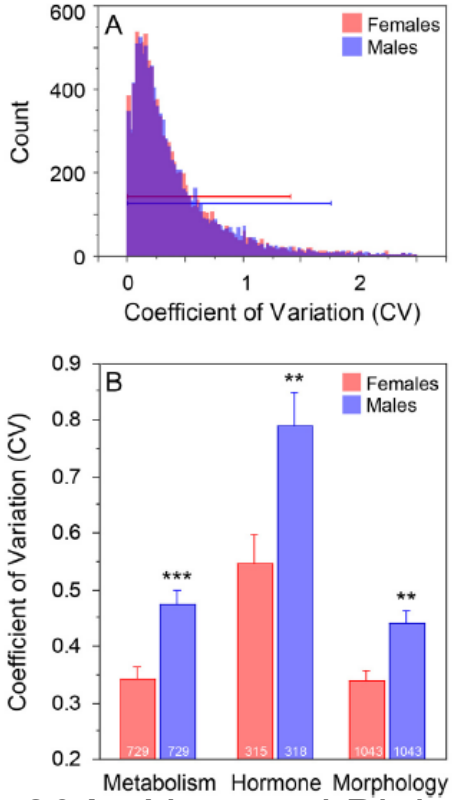
Experiments isolate cause and effect by simplification

	Standard	Contemporary
Reduction	Methods which minimise the number of animals used per experiment	Appropriately designed and analysed animal experiments that are robust and reproducible, and truly add to the knowledge base

https://www.nc3rs.org.uk/the-3rs

Misconception: ♀ hormonal cycles = more variable

- meta-analysis 293 published articles
- female mice tested at random stages of the estrous cycle were compared with males
- behavioral, physiological,morphological, and molecular traits



Prendergast **2014** Neurosci Biobehav Rev

Skill gap - analysis gets more complex

One sex:

- Student's t-Test
- Y ~ treatment

Both sexes:

- Two way ANOVA
- Y ~ sex + treatment + sex*treatment

- 4/10 Americans hated maths (IPOS 2005 study)
- Mathematical anxiety recognised psychological condition.
- 28% fewer citations for each additional equation per page in the main text (Fawcett 2013 PNAS)

Misconception: It will DOUBLE my animal usage

“Keep doing what you are already doing but change half the animals in your study to female”

*McCarthy **2015** Schizophrenia Bulletin*

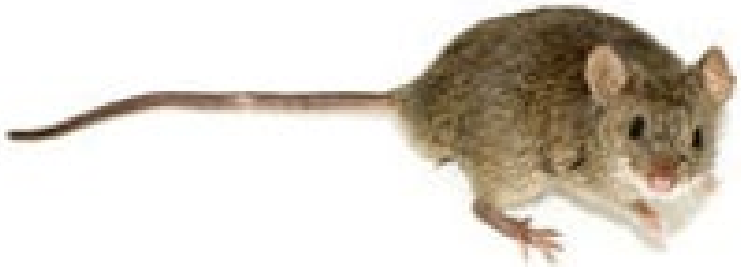
In terms of statistical power:

Y ~ Treatment ➡ Y~ Treatment + Sex+ Sex*Treatment

Practical issues increase complexity

Caged by sex

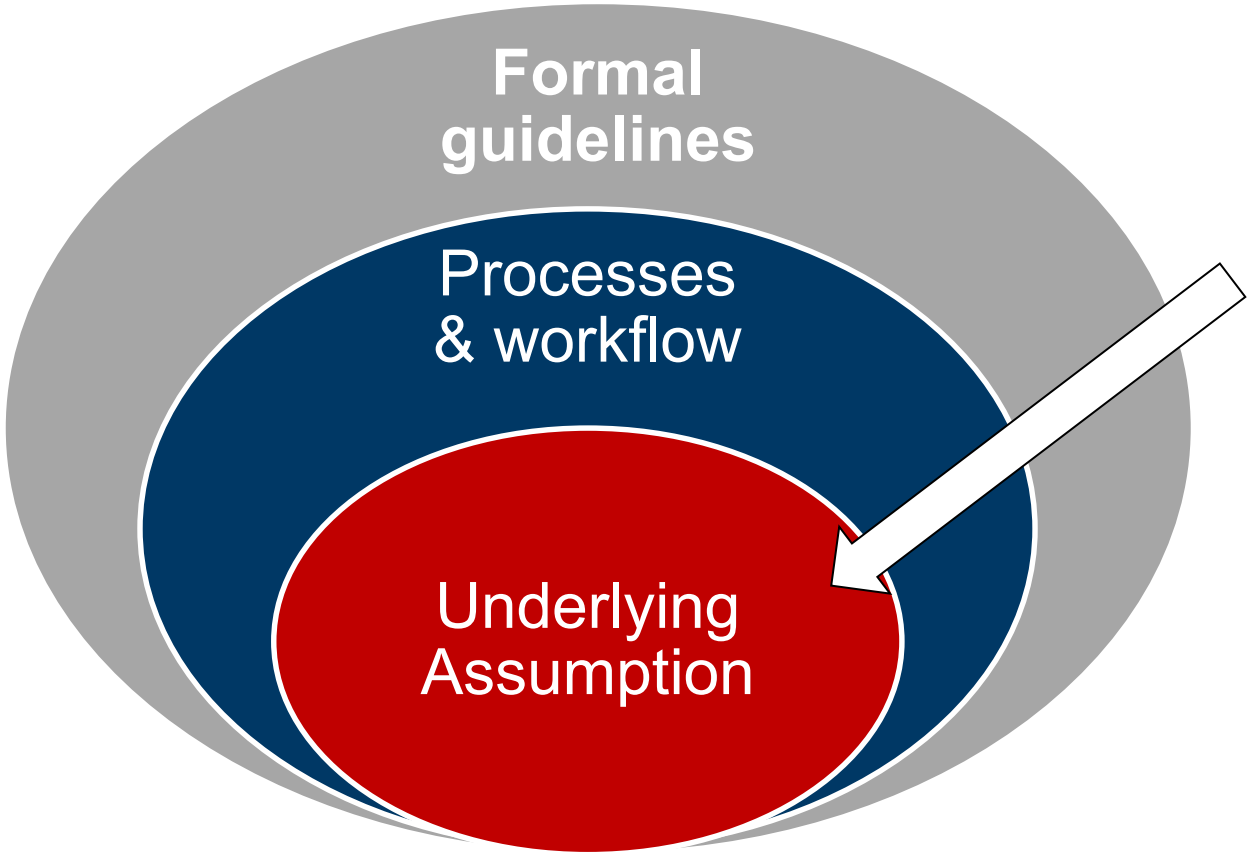
Pheromones



Order effects?

Clean equipment between sexes?

Culture is driven by the underlying unconscious beliefs



SCHEIN, E. H. 2010 Onion Model

- It is not possible to include both sexes. I couldn't justify it.
- There is no benefit to include women

British Journal of Pharmacology

British Journal of Pharmacology (2018) •• ••–•• 1

REVIEW ARTICLE THEMED ISSUE

Sex bias in preclinical research and an exploration of how to change the status quo

Correspondence Natasha A. Karp, Quantitative Biology, Discovery Science IMED Biotech Unit, AstraZeneca Darwin Building (Unit 310), Cambridge Science Park, Cambridge CB4 0WG, UK. E-mail: natasha.karp@astrazeneca.com

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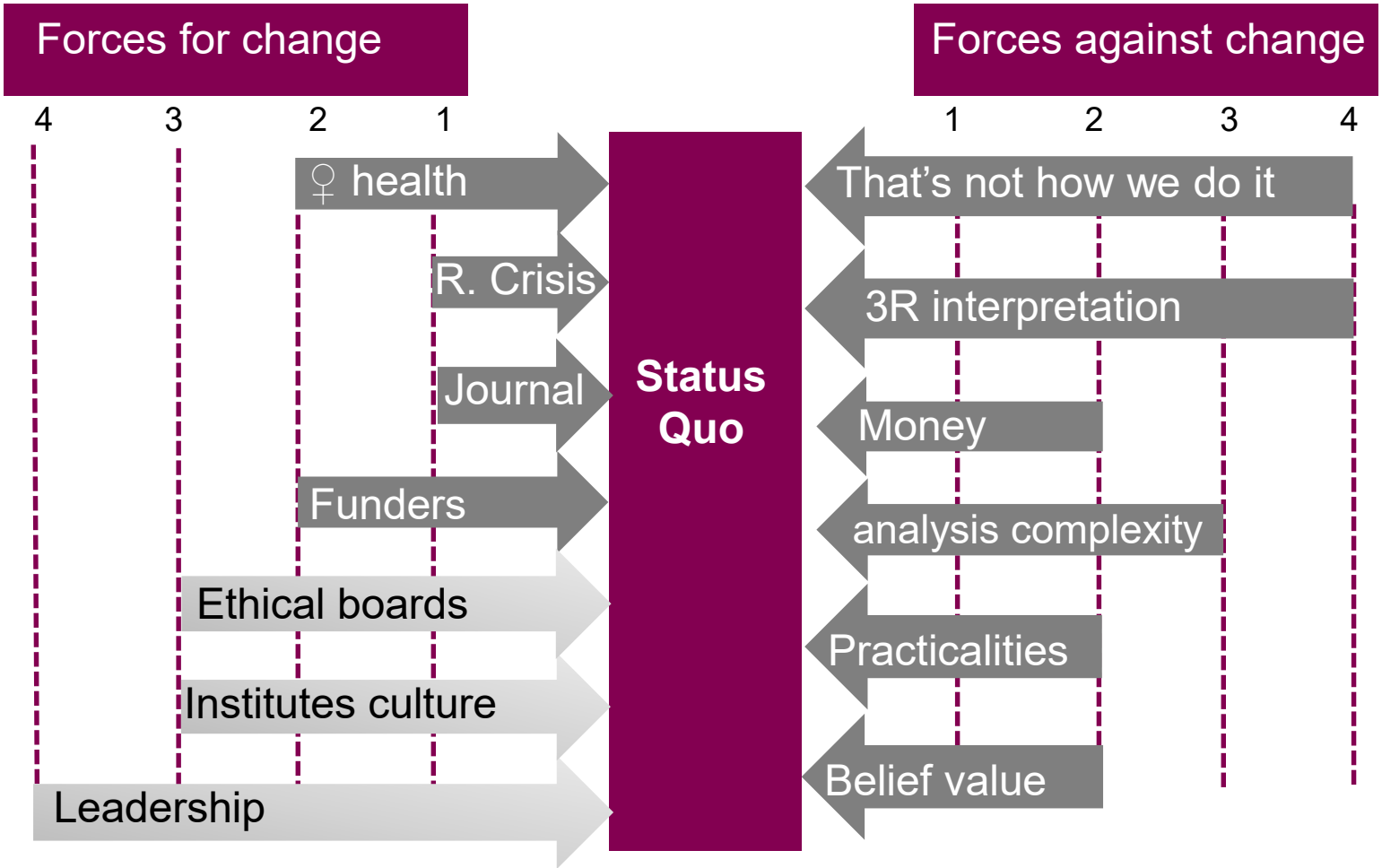
Natasha A Karp[1] and Neil Reavey[2,3]

Force field analysis



Conclusions

- Sex bias is culturally embedded in our research pipelines, impacting the reporting, design, and analysis.
- Precision medicine typically focuses on differences in genetic factors, environment or lifestyle. Considering the sex of the patient provides an easy win to optimising the healthcare.
- Research suggests that sex is a significant source of variation for both *in vivo* and *in vitro*.
- This isn't an individual scientists issue but a scientific practice issue
- We need to consider this as a change management issue
  - We need to win the hearts and minds of the individuals
  - Focus on removing resisting forces and strengthen driving forces.

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