Sex bias in preclinical studies

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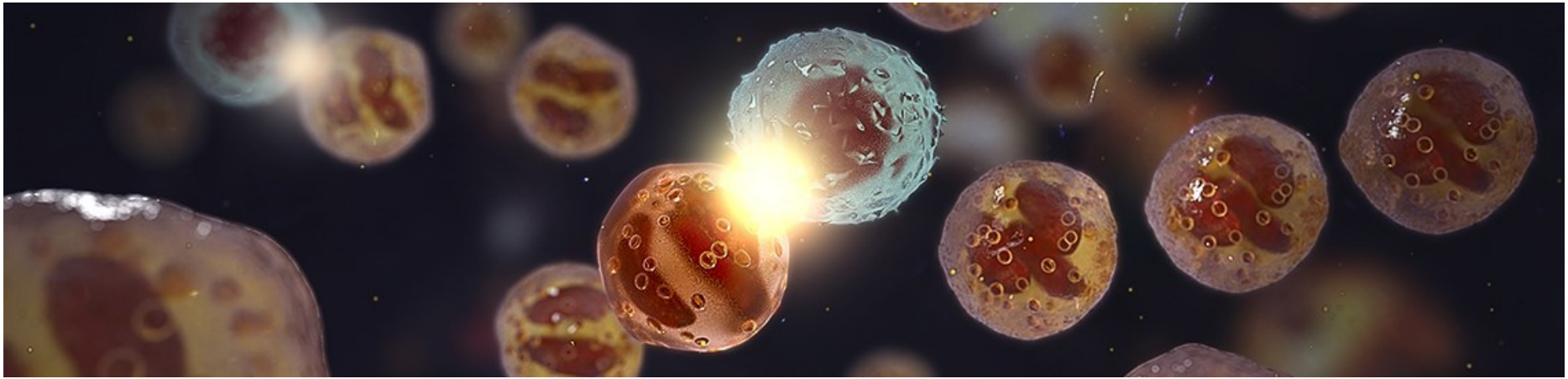


Talk outline

- Call to consider sex as a biological variable (SABV)
- Prevalence of SABV
- Why a sex bias exist?
- Sociological exploration of the topic
- Challenge of change

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

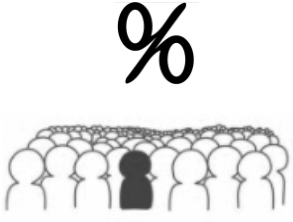
    Mazure and Jones 2015 BMC Women's health
- **Analysis**:
  - When both sexes, only 33% analysed by sex

    Beery and Zucker 2011 Neurosci Biobehav Rev

What about ♀ prevalent disease?

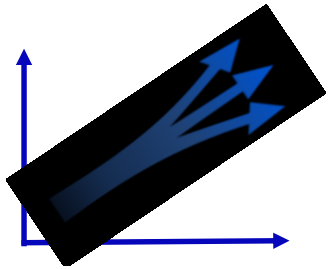
- Yoon *et al* 2014 Surgery
- Reviewed surgical biomedical research published 2011-2012
- For publications on ♀ prevalent diseases (n=45)
  - 44% did not report the sex studied
  - For those that reported the sex, only 12% studied ♀

Personalised medicine: sex

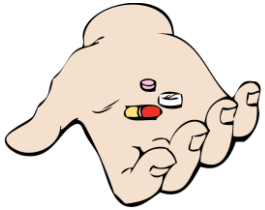


Prevalence

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- 8/10 drugs withdrawn from the US market from 1997 to 2000 posed greater health risks for women than for men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

What is the expectation?

- “report their plans for the balance of male and female cells and animals in preclinical studies in all future application, unless sex specific inclusion is unwarranted, based on rigorously defined exceptions”

- Note: Not the identification of sex effects

Exceptions?

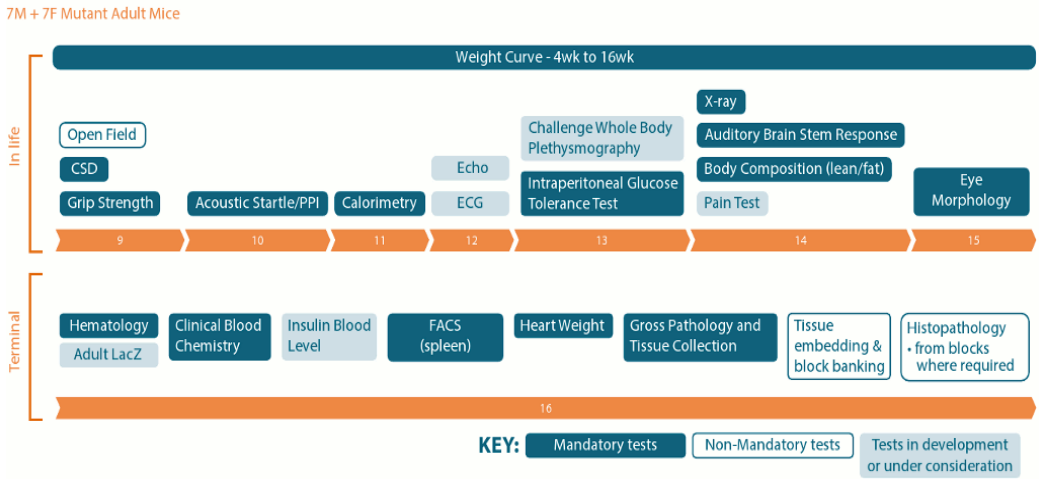
- Pure molecular studies such as P-P interactions
- Sex-specific conditions or phenomena e.g. ovarian cancer
- Acutely scare resources (e.g. non human primates)
- If you can provide strong justification.
- Absence of evidence regarding sex differences is not justification

Controversy

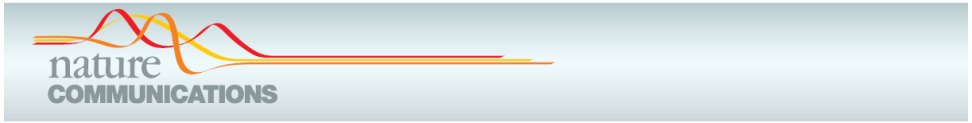
- Field, R **Nature** 2014
  - “major step in the wrong direction” , “Waste of resources”
- Sandberg, K **Am J Physiol Regul Integr Comp Physiol** 2014
  - Unnecessary duplication and slow progress.
- Richardson **PNAS** 2015
  - question value of preclinical research in to sex differences
- Ritz **FASEB 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

International Mouse Phenotyping Consortium



Published study – prevalence of sexual dimorphism



ARTICLE

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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.

Assessing the role of sex in an individual experiment

SABV within control data

Control data for an institute

Statistical pipeline

How often was sex a significant source of variation?

As a modifier of the treatment effect

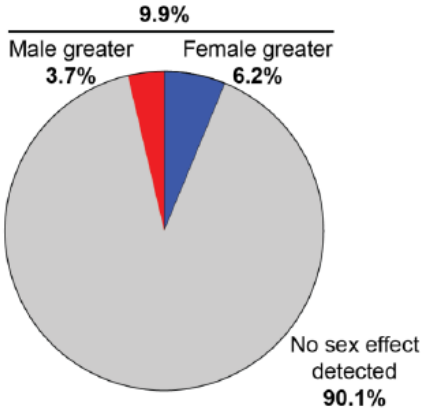
Control and treated data from an institute for variable of interest

Statistical pipeline

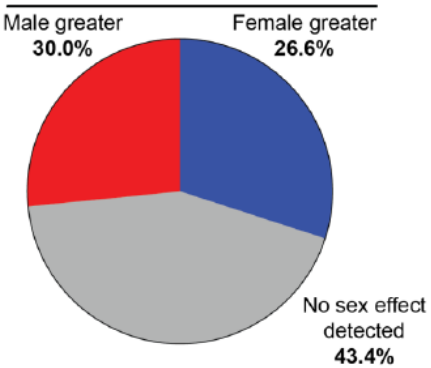
How often was treatment effect dependent on the sex?

SABV?

In control data

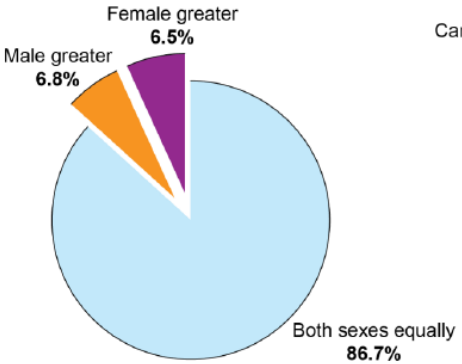


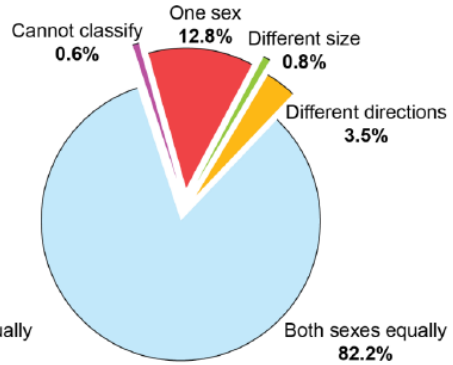
Categorical
N=545



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

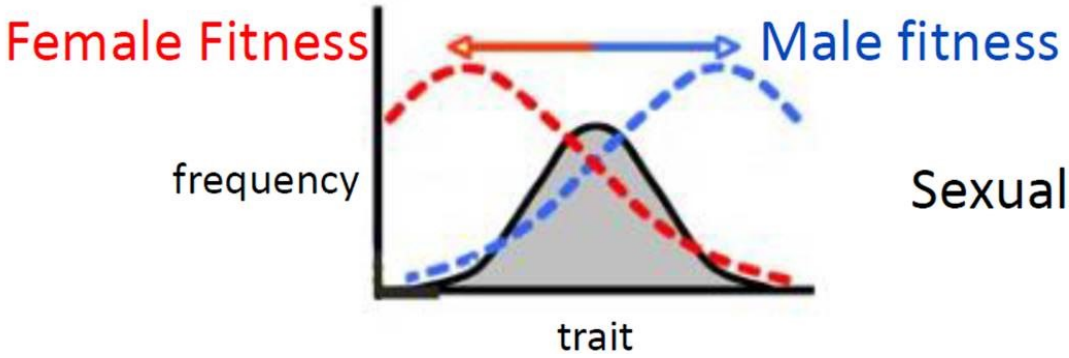
Karp et al 2017 Nature Communications

WHY DOES A SEX BIAS EXIST?

- MISCONCEPTIONS
- SKILL GAP
- PRACTICAL CONCERNS
- 3R INTREPRETATION

Concept drivers of sex difference evolved

Continuum → Hormonal differences → Driven molecular differences → Driven by chromosome differences



Sexual conflict in evolution

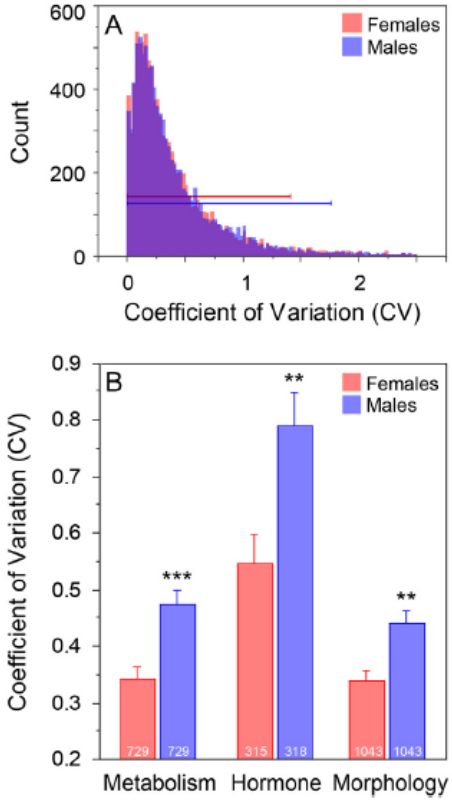
In-vivo impacted by the ethical framework

	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

Assumption: ♀ 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

- Concurrent design studying continuous measure
- Interest: impact on mean

One sex:

- Student's t-Test
- Y ~ treatment

Two sexes:

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

Fear of statistics?

- 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)

- Culturally you are outlier if you like maths. It is almost said as a badge of honour that you don’t gets stats.

Misconception: It will increase my animal usage

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

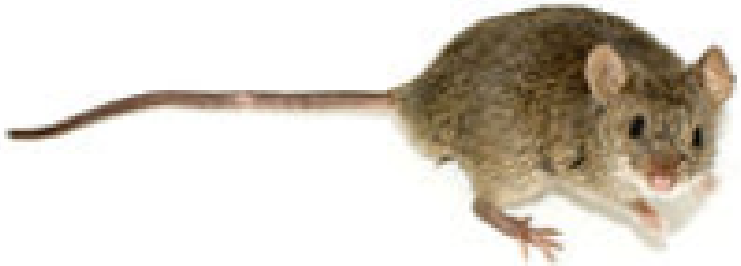
*McCarthy **2015** Schizophrenia Bulletin*

In terms of statistical power:

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

Practical issues increase complexity

Caged by sex



Pheromones

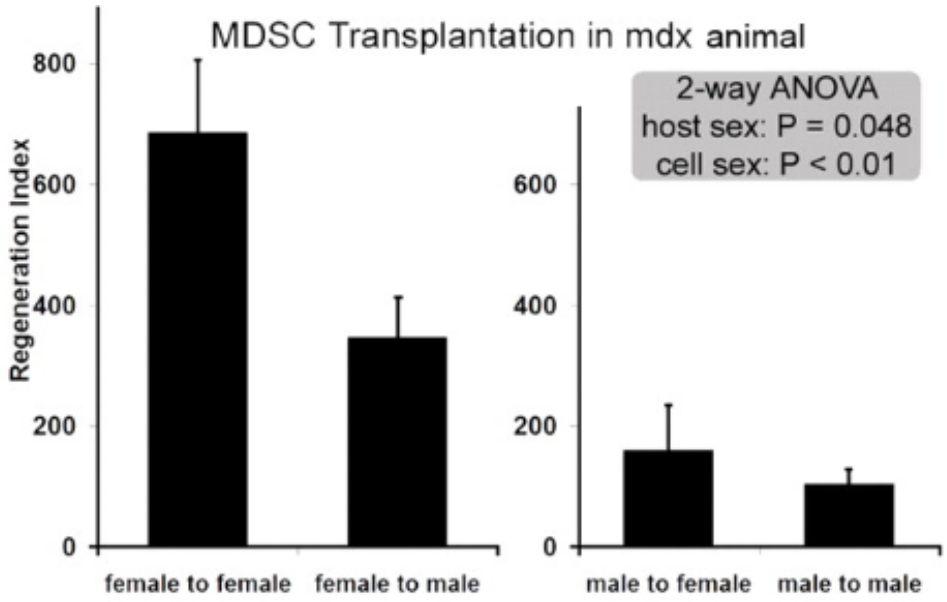
Order effects?

Clean equipment between sexes?

In vitro misconception

- Belief: sex of the cell is irrelevant
- Sex differences predominately derived from hormonal differences
- Often the sex isn't know
- However ….. Shah 2014 Do you know the sex of your cells?

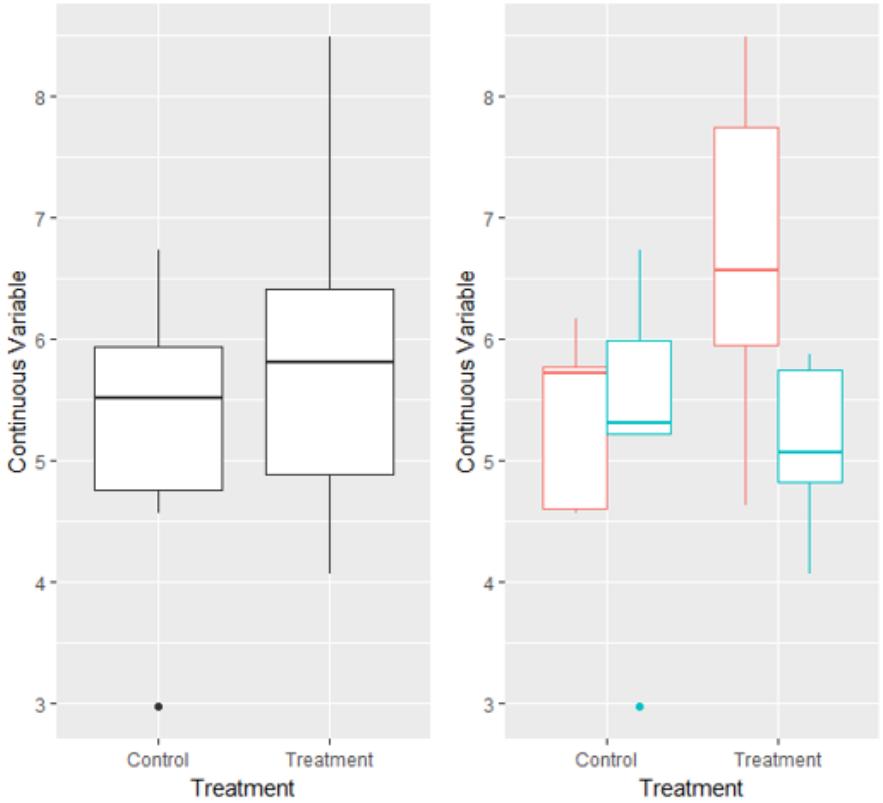
Example impact of cell's sex



- Shown that muscle-derived stem cells transplanted into dystrophic (*mdx*) mice efficiently regenerate skeletal muscle
- But heterogeneity in response muscle-derived stem cells
- Cell sex had a considerable impact
- Proposed this difference may arise from innate sex-related differences in the cells' stress responses.

Deasy 2007 JCB A role for cell sex in stem cell–mediated skeletal muscle regeneration: female cells have higher muscle regeneration efficiency

Example impact pooling



CHALLENGE OF CHANGE

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

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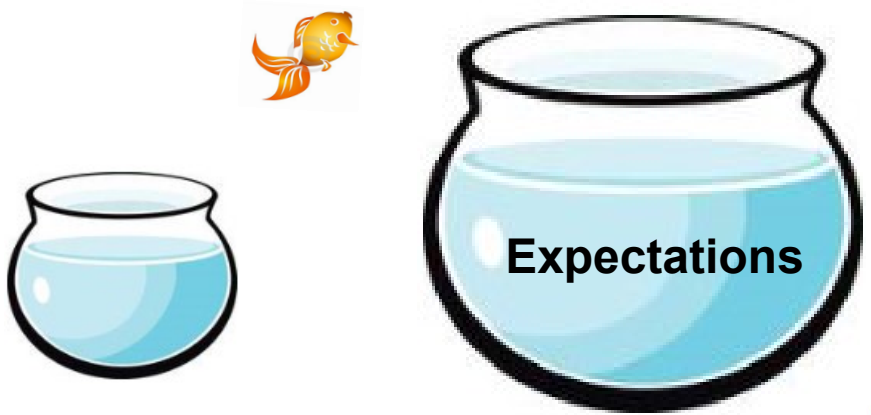
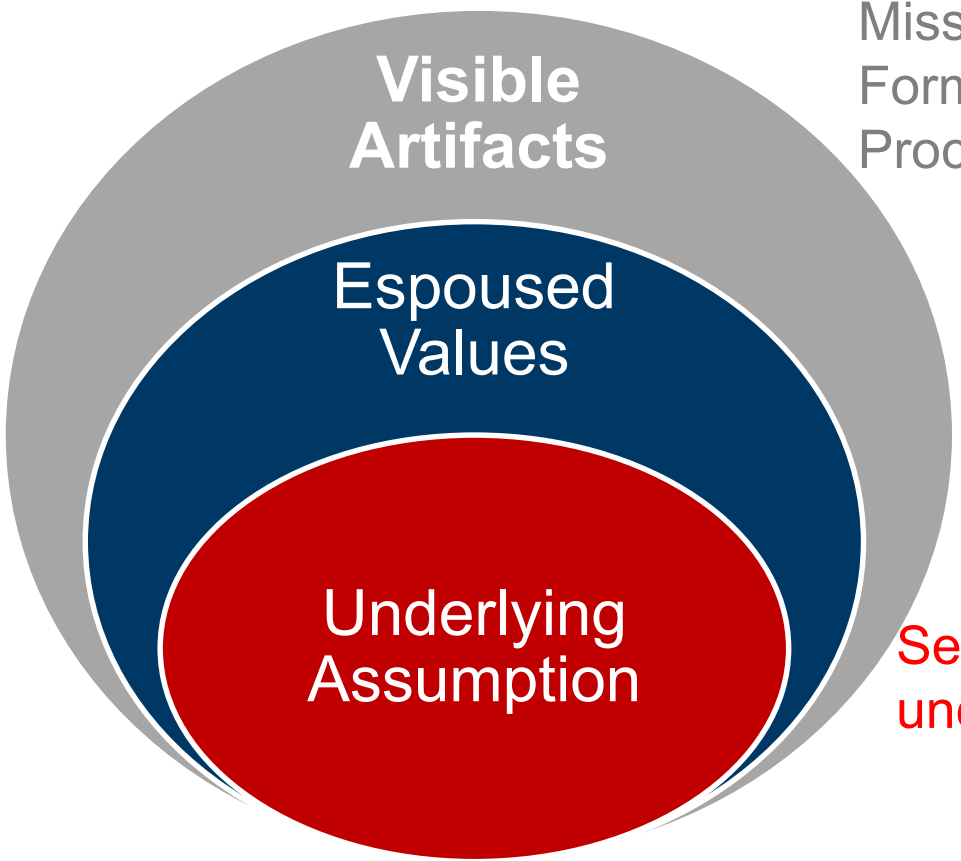
Natasha A Karp[1] and Neil Reavey[2,3]



Organisational Culture ‘the way we do things around here”.



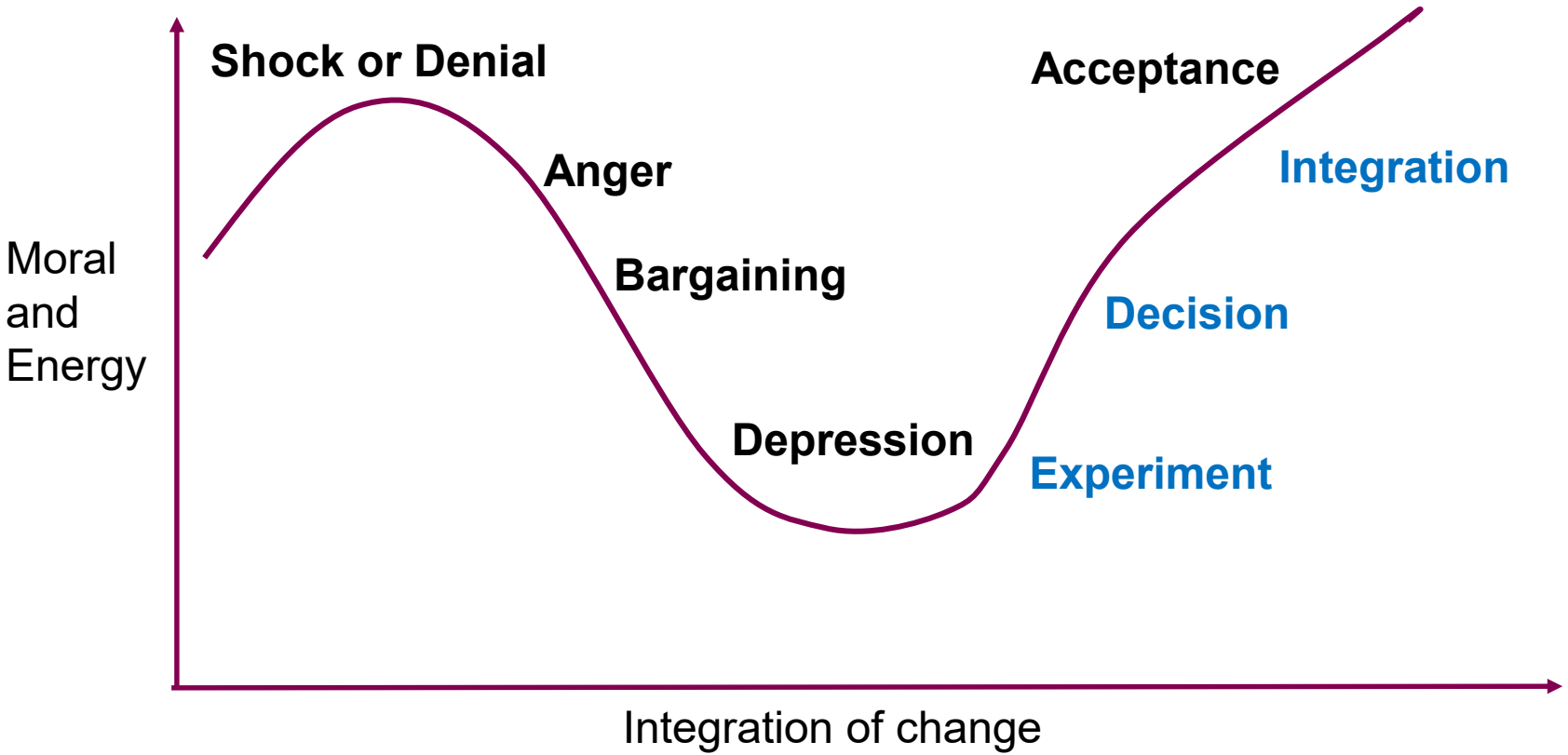
Mission/vision statement
Formal guidelines
Processes

Rules of conduct
Values
Standards

Self evident and unconscious behaviour

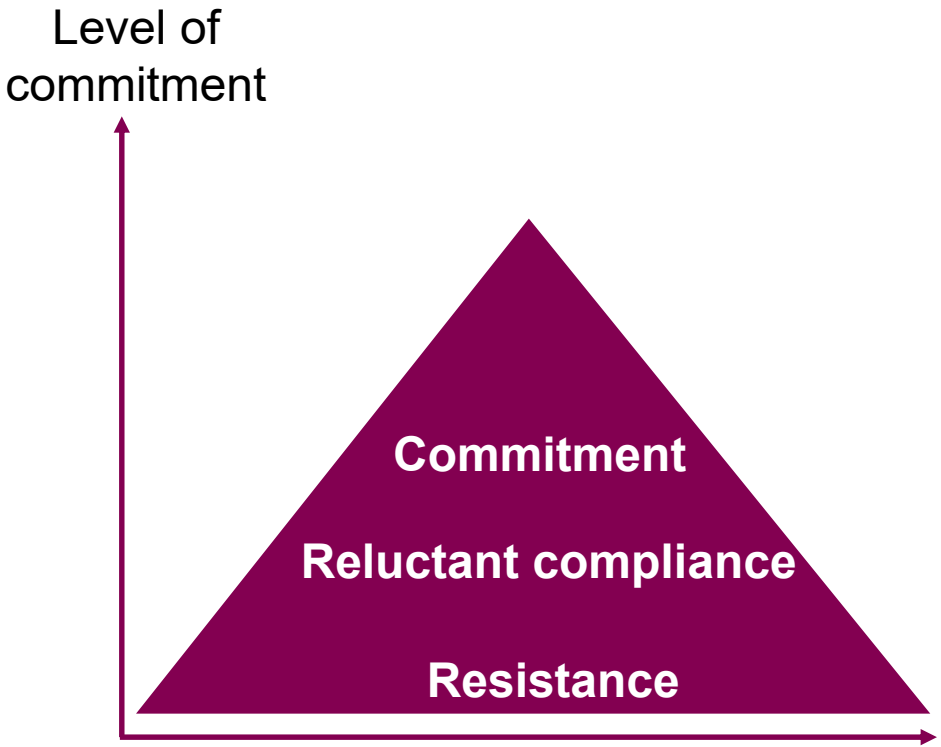
SCHEIN, E. H. 2010 Organisation Culture and Leadership

Küber-Ross Change Curve



Scire, P. 2007 "Applying Grief Stages to Organizational Change."

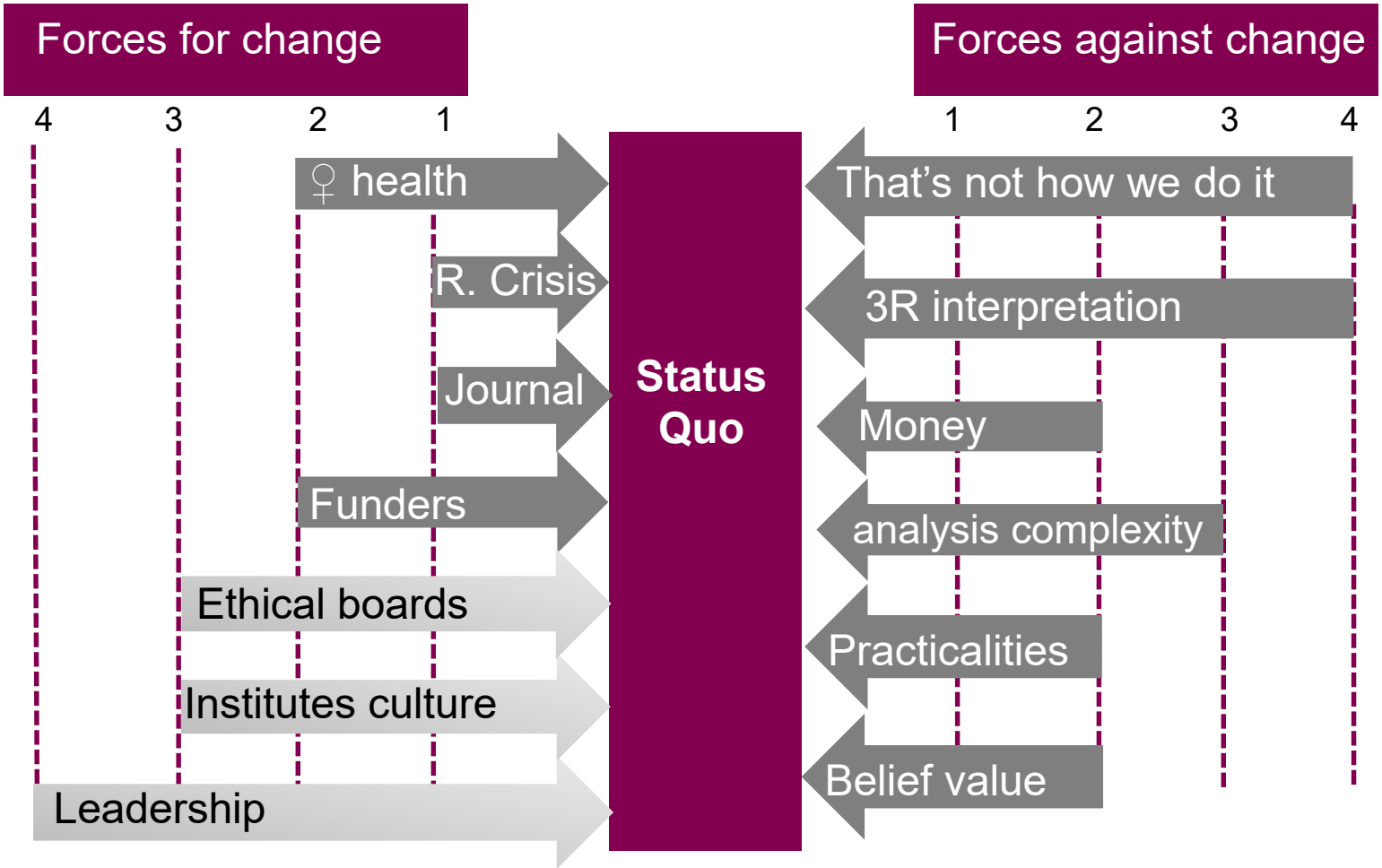
Influence tactics



Tactics	
Positive	Rational Persuasion
	Exchange
	Consultation
	Inspirational Appeal
Negative	Pressure
	Legitimising
	Coalition Building

Gary Yukl 1981 Leadership in organization

Force field analysis



Institute level plan

	Kotter - 8 step change process
Leadership	1: Create Urgency
	2: Form a Powerful Coalition
	3: Create a Vision for Change
	4: Communicate the Vision
Management	5: Remove Obstacles
	6: Create Short-Term Wins
	7: Build on the Change
	8: Anchor the Changes in the Culture

John Kotter 1995 Leading Change

SOCIOLOGICAL EXPLORATION OF THE ISSUE

- Interviews to probe scientists' (n=9) thoughts and experiences
- *E.g.*
  - *Do they think about SABV in their research?*
  - *Do they think sex differences are important to health and their research?*
- Qualitative research looking for patterns of meaning "themes"

Annirka Gomper
Master dissertation 2019 Cambridge University

Conflicting themes identified

Generalizability

- Important to embrace variation to understand biological differences

Avoiding complexity

- To make progress in science reduce complexity

Practicality

- Mediate tension between generalizability and avoiding complexity
- Convert the original research question into a doable problem
- Avoid looking for sex differences as want a simple answer as won’t be able to practically address them

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
- How do we support scientists to be more mindful of sex in our research pipeline?

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