Systematic reviews of experimental animal studies

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Collaborative Approach to Meta-Analysis and Review of
Animal Data from Experimental Studies

and

University of Edinburgh

CAMARADES: Bringing evidence to translational medicine

Disclosures

- Member of UK Home Office Animals in Science Committee (Convener of 3Rs subgroup)

- Member, UK Commission for Human Medicines, MHRA

- Have sought and will seek funding for work in this area

Translational Medicine 101

Definition: using information from one research domain to guide research in a different research domain

Context: Many proposals for clinical trials claim some justification from supporting animal data

Neural Correlates of Interspecies Perspective Taking in the Post-Mortem Atlantic Salmon: An Argument For Proper Multiple Comparisons Correction

Craig M. Bennett[1*], Abigail A. Baird[2], Michael B. Miller[1] and George L. Wolford[3]

One mature Atlantic Salmon (Salmo salar) participated in the fMRI study. The salmon measured approximately 18 inches long, weighed 3.8 lbs, and was not alive at the time of scanning. It is not known if the salmon was male or female, but given the post-mortem state of the subject this was not thought to be a critical variable.
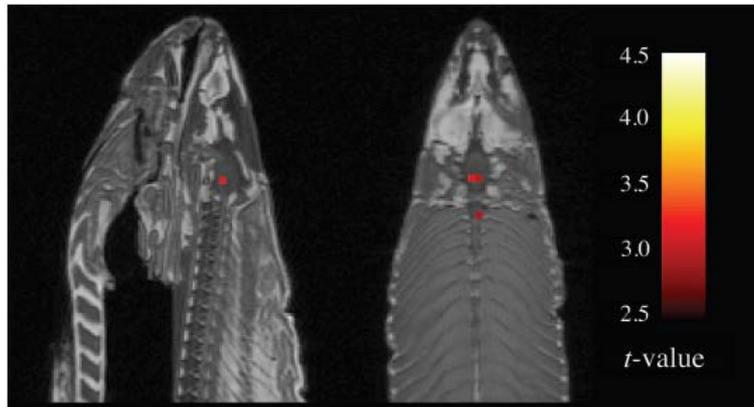
The task administered to the salmon involved completing an open-ended mentalizing task. The salmon was shown a series of photographs depicting human individuals in social situations with a specified emotional valence, either socially inclusive or socially exclusive. The salmon was asked to determine which emotion the individual in the photo must have been experiencing.

Several active voxels were observed in a cluster located within the salmon's brain cavity (see Fig. 1). The size of this cluster was 81 mm^3 with a cluster-level significance of $p = 0.001$.

Either we have stumbled onto a rather amazing discovery in terms of post-mortem ichthyological cognition, or there is something a bit off with regard to our uncorrected statistical approach.

Winner of the 2012 Ignoble Prize for Neuroscience

Systematic review

- A review article where criteria for identifying and considering information are determined in advance and are transparent

- Contrasts with, – and is less biased than – narrative reviews

- Provides additional insights to assessments of "biological truth"

Systematic reviews v narrative reviews

- House dust mites and asthma
  - 63 of 70 review articles claimed efficacy for physical eradication measures (vacuum cleaning, bed covers, freezing …)
  - Most frequently cited study had 7 patients per group
  - Systematic review (Cochrane) identified 28 trials involving 939 patients
    - Found no effect of physical measures in improving outcome

Schmidt and Gotzsche, 2005 J Fam Practice

"Authors often use non randomised studies to create a false impression of consensus"

REVIEW ARTICLE

Systematic reviews and meta-analysis of preclinical studies: why perform them and how to appraise them critically

Emily S Sena[1,2], Gillian L Currie[1], Sarah K McCann[2], Malcolm R Macleod[1] and David W Howells[2]

Table 2. Guidelines for reporting systematic reviews and meta-analyses of animal studies

Title	Identify the report as a systematic review and/or meta-analysis of animal experiments.
Abstract	Provide a structured abstract covering the following: objectives, data sources, review methods, results, and conclusion.
Introduction	Clearly defined and focussed research question.
Methods	
Protocol	Indicate if a protocol exists and where it can be found (i.e., web address).
Searching	Describe the information sources in detail, including keywords, search strategy, any restrictions, and special efforts to include all available data.
Selection	Describe the inclusion and exclusion criteria.
Validity and quality assessment	Describe the criteria and process used to assess validity.
Data abstraction	Describe the process or processes used (e.g., completed independently, in duplicate). Describe whether aggregate data or individual animal data are abstracted.
Study characteristics	Describe the study characteristics relevant to your research question.
Quantitative data synthesis	Describe the principal measures of effect, method of combining results, handling of missing data; how statistical heterogeneity was assessed; and any assessment of publication bias—all in enough detail to allow replication.
Results	
Flow chart	A meta-analysis profile summarizing study flow giving total number of experiments in the meta-analysis.
Study characteristics	Descriptive data for each experiment.
Quantitative data synthesis	Present simple summary results (e.g., forest plot); identify sources of heterogeneity, impact of study quality, and publication bias.
Discussion	Summarize the main findings; discuss limitations; provide general interpretation of the results in the context of other findings, and implications for future research.
Funding	Describe sources of funding for the review and other support. The role of funders should be presented.
Conflict of interest	Any potential conflict of interests should be reported.

Meta-analysis

- A statistical technique to combine data from separate experiments
  - To give an overall "best estimate" of a biological effect
  - To understand the impact of other things on that effect
    - Related to the exposure (dose, time …)
    - Related to the animal (age, sex, comorbidity …)
    - Related to experimental design

Clinical Neuroscience
Invited review

Meta-analysis of data from animal studies: A practical guide☆

H.M. Vesterinen [a], E.S. Sena [a,b], K.J. Egan [a], T.C. Hirst [a], L. Churolov [b], G.L. Currie [a],
A. Antonic [b], D.W. Howells [b], M.R. Macleod [a,*]

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[b] The Florey Institute of Neuroscience and Mental Health, University of Melbourne, Australia
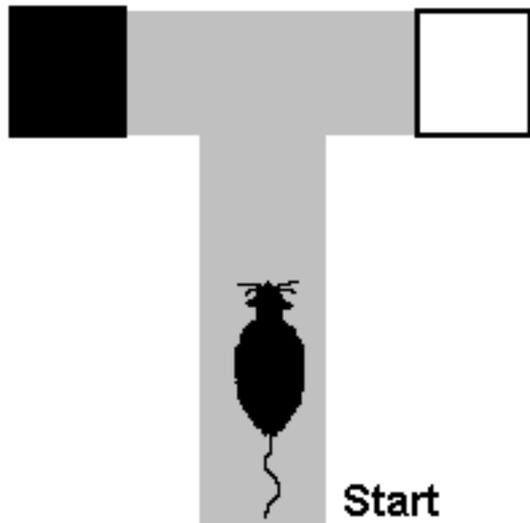
You can usually find what you're looking for …

- 12 graduate psychology students
- 5 day experiment: rats in T maze with dark arm alternating at random, and the dark arm always reinforced
- 2 groups – "Maze Bright" and "Maze dull"

Start

Group	Day 1	Day 2	Day 3	Day 4	Day 5
"Maze bright"	1.33	1.60	2.60	2.83	3.26
"Maze dull"	0.72	1.10	2.23	1.83	1.83
Δ	+0.60	+0.50	+0.37	+1.00	+1.43

Rosenthal and Fode (1963), Behav Sci 8, 183-9

Bias in ischaemia studies

- Infarct Volume
  - 11 publications, 29 experiments, 408 animals
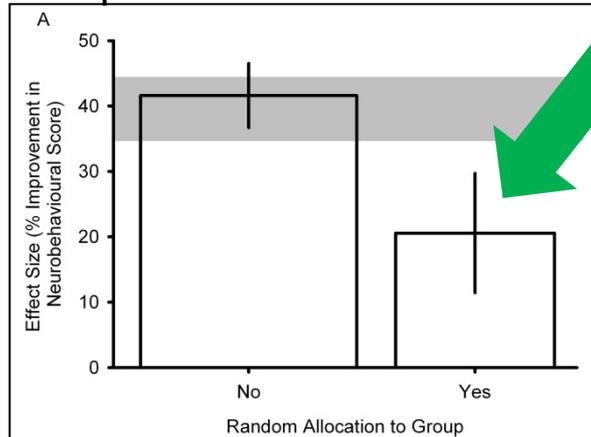 - Improved outcome by 44% (35-53%)

Randomisation   **Blinded conduct of experiment**   **Blinded assessment of outcome**
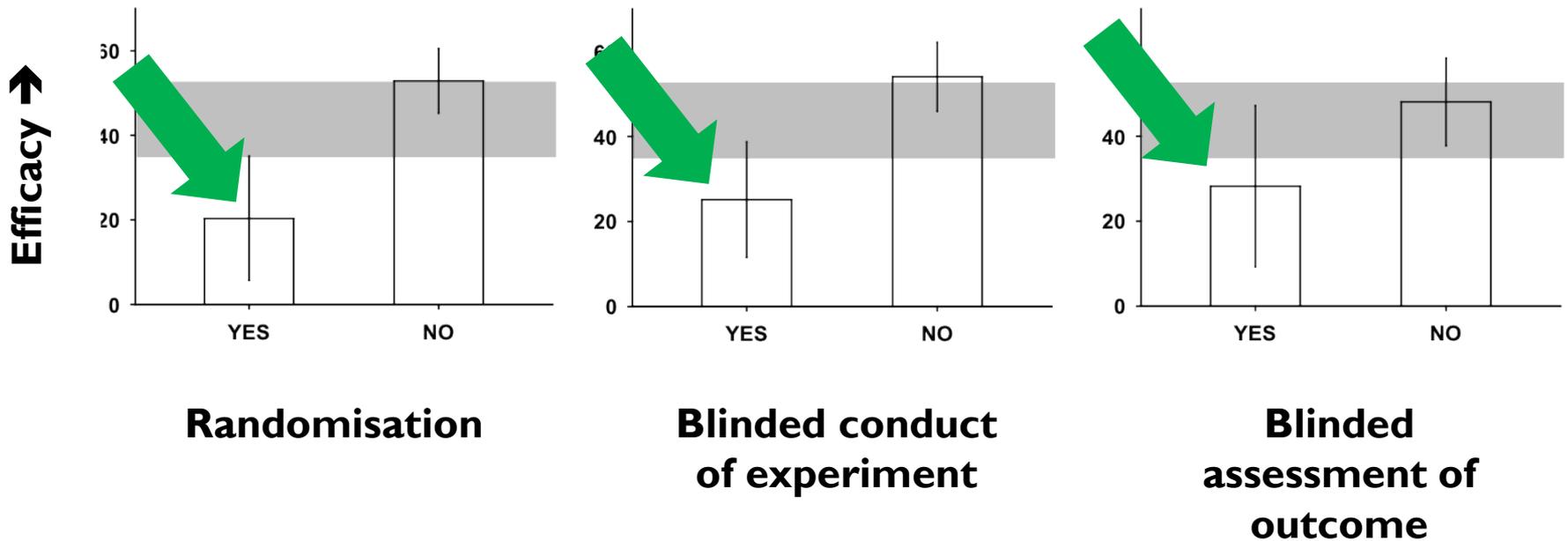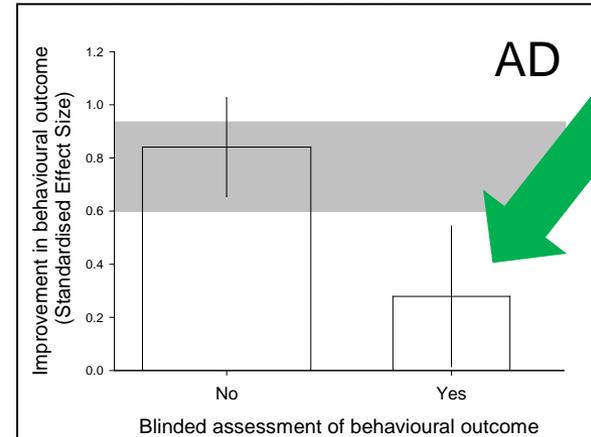
Macleod et al, 2008
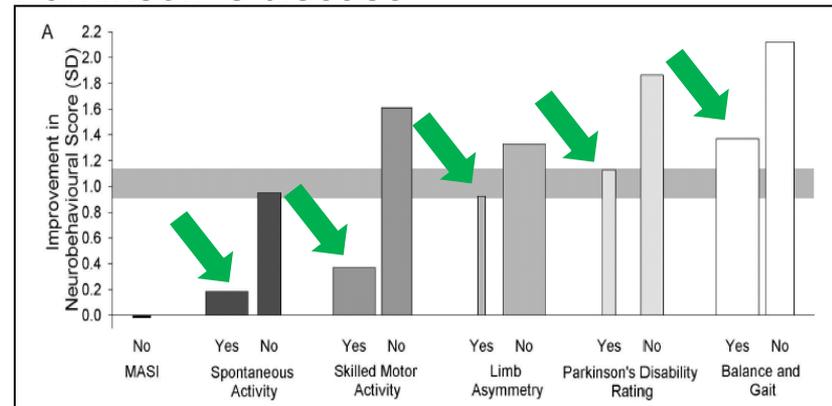
Lessons from other neuroscience domains

Multiple Sclerosis
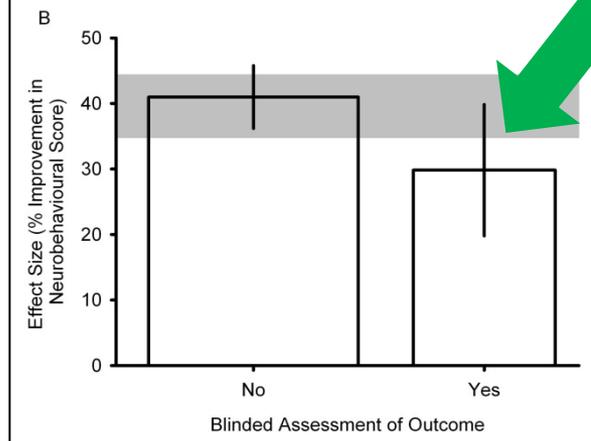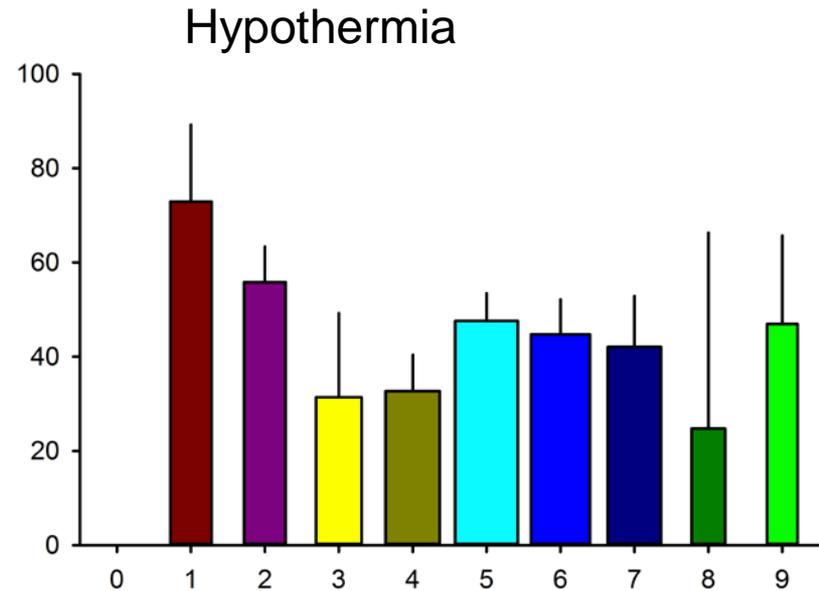

Alzheimer´s disease
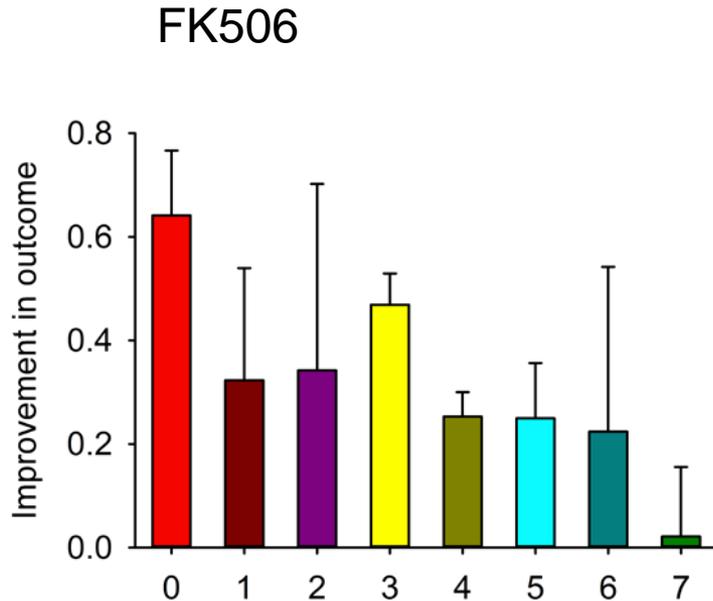

Parkinson´s disease

Associations between quality of reporting and observed effect sizes
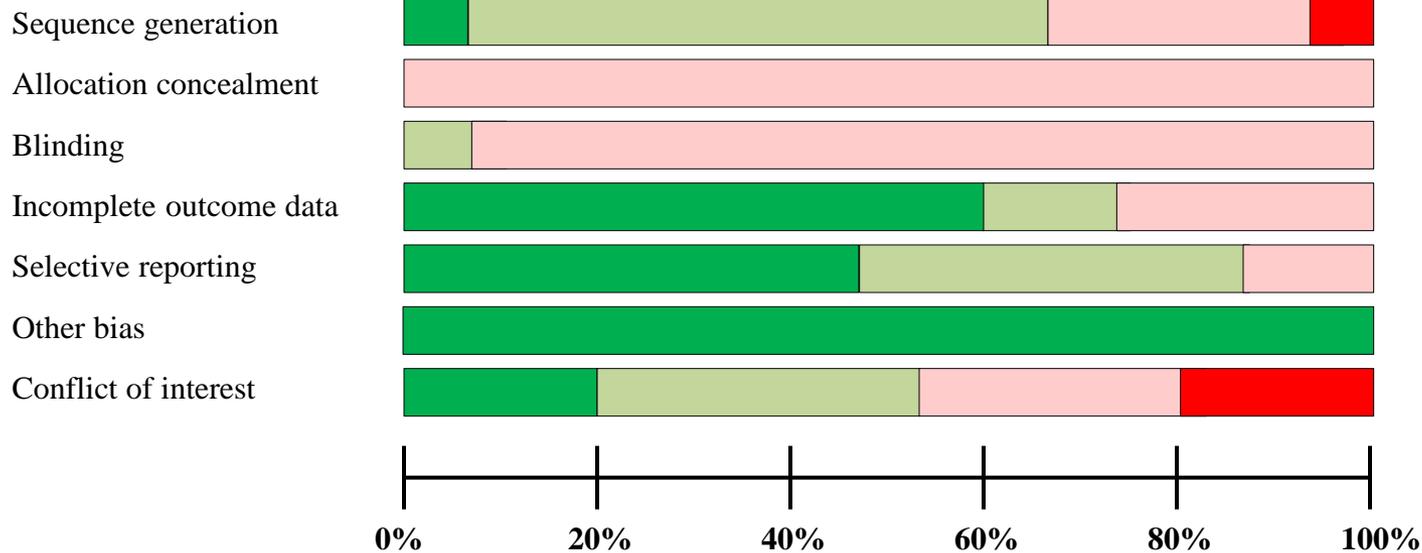
FK506

Hypothermia

Number of study quality checklist items scored

Risk of Bias

Slide from Tracey Woodruff/
Navigating the Science

The scale of the problem
RAE 1173

"an outstanding contribution to the internationally excellent position of the UK in biomedical science and clinical/translational research."
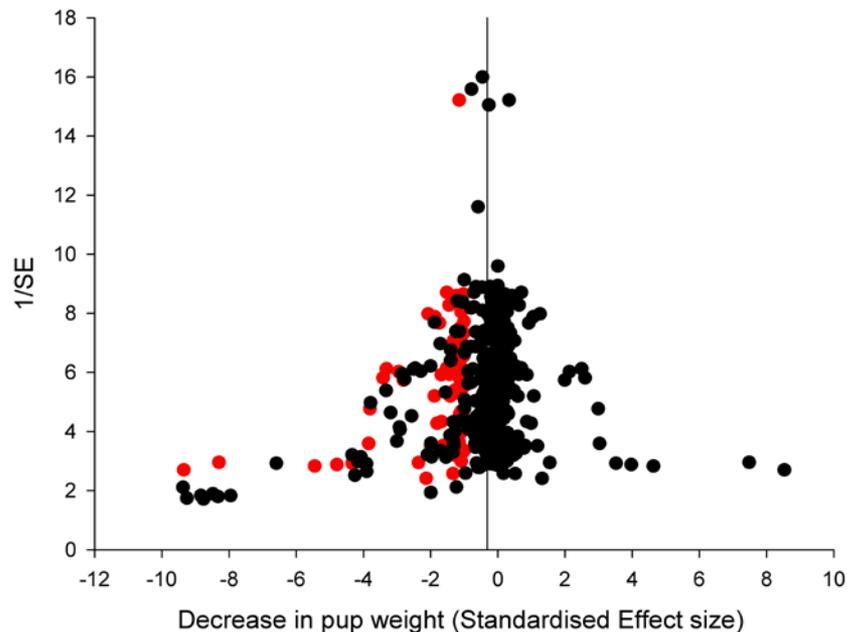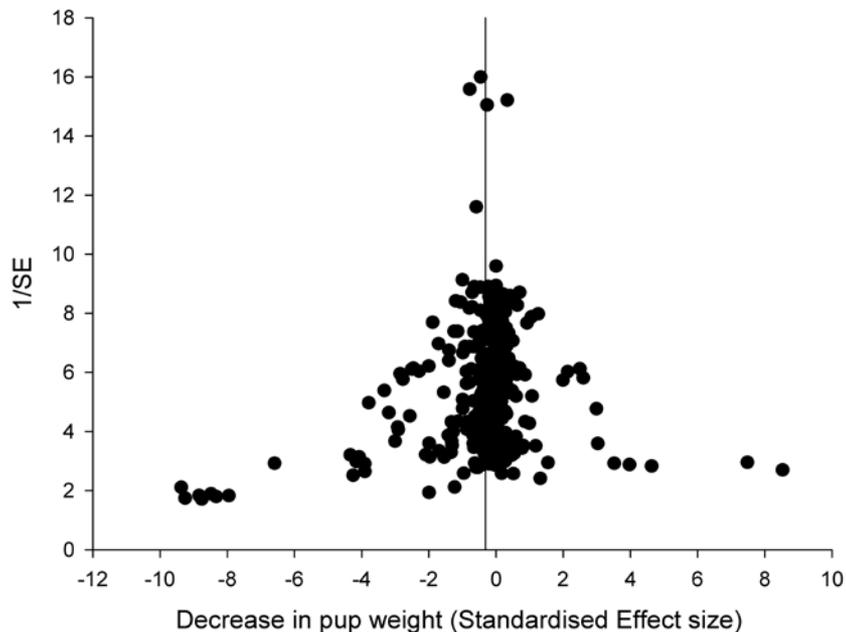
"impressed by the strength within the basic neurosciences that were returned …particular in the areas of behavioural, cellular and molecular neuroscience"

1173 publications using non human animals, published in 2009 or 2010, from 5 leading UK universities

CAMARADES: Bringing evidence to translational medicine
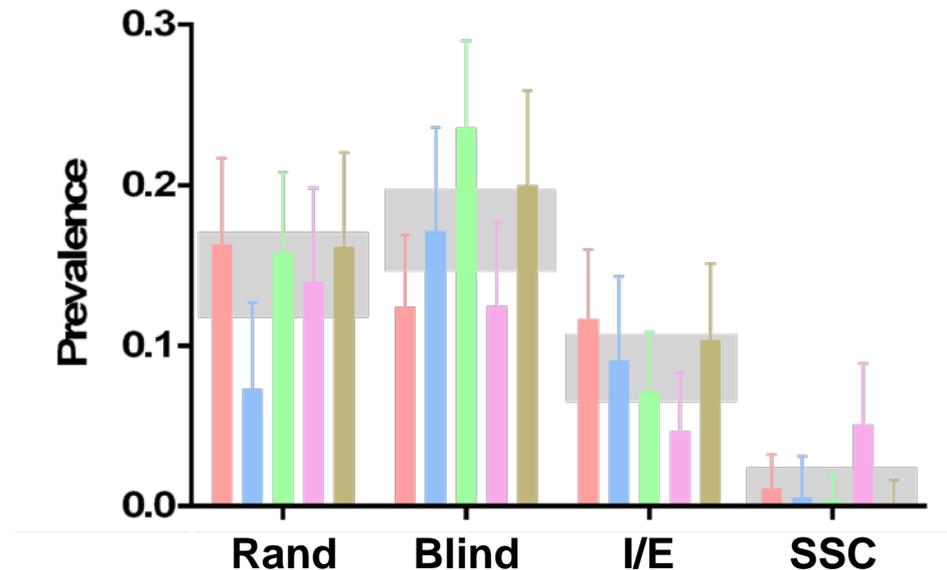
Publication bias in toxicology studies

Different patterns of publication bias in different fields

	outcome	observed	corrected	
Disease models	improvement	40%	30%	Less improvement
Toxicology model	harm	0.32	0.56	More harm

Harm   Benefit
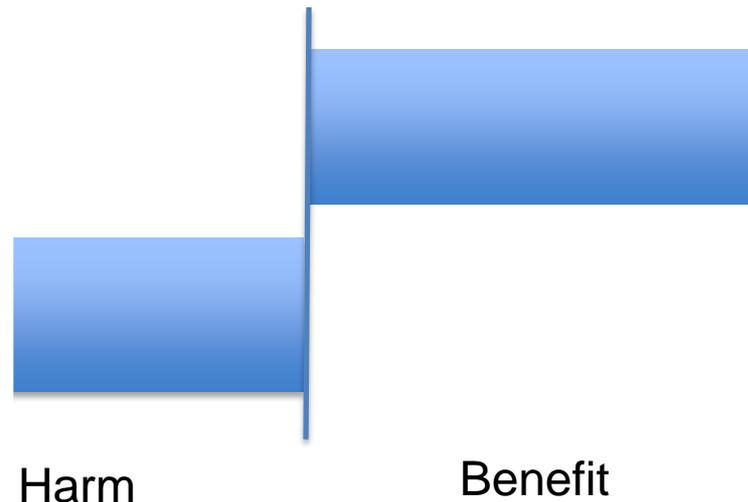
How big a hole does this make in published research?

Risk of Bias

Publication Bias

Underpowered experiments
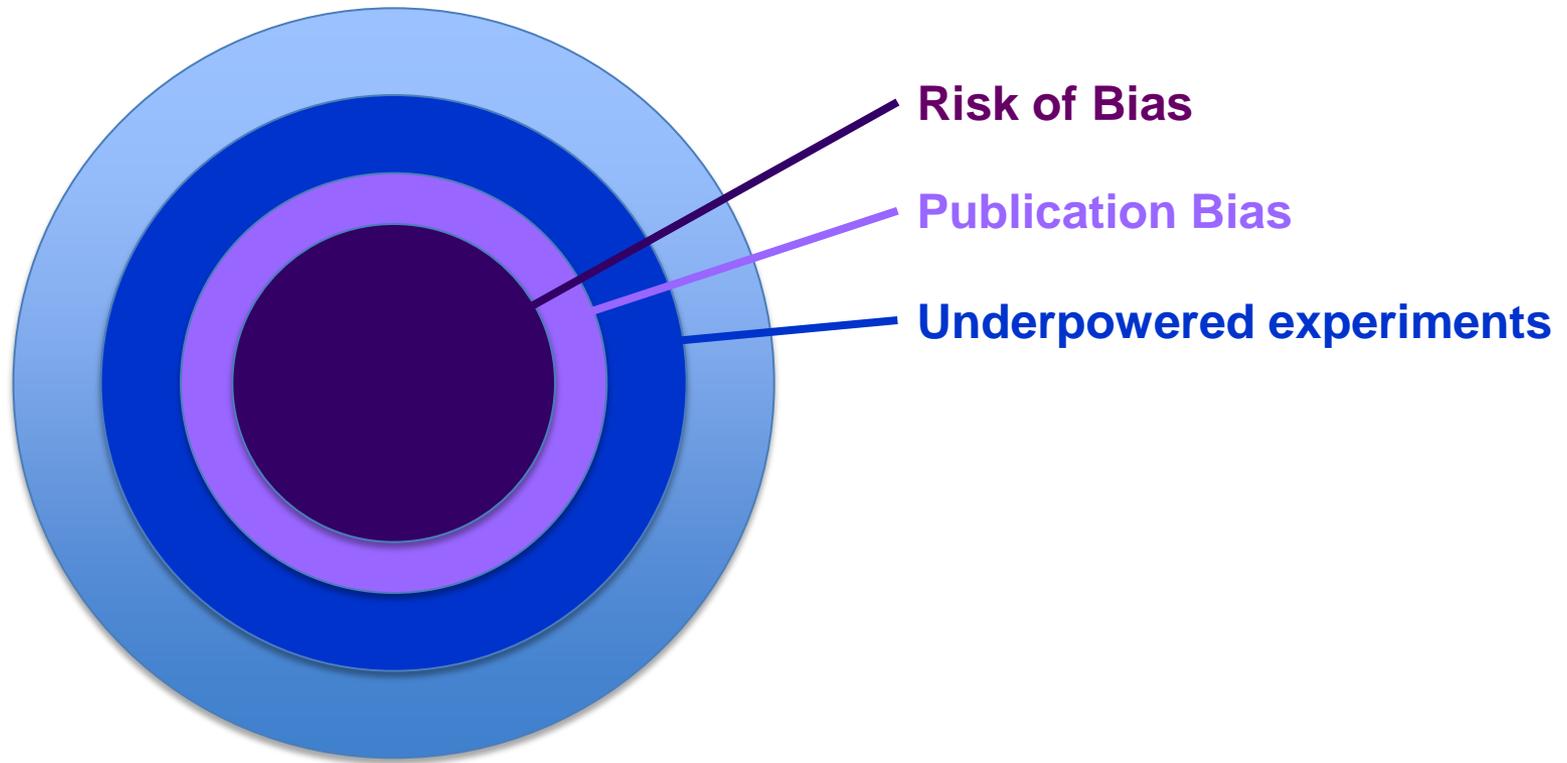
Technical reflections

- The most important thing we will find out is about risk of bias in this research domain

- Fixed effects v random effects

- Weighted mean difference v standardised mean difference

- Stratified meta-analysis v meta-regression

- Univariate v multivariate meta-regression
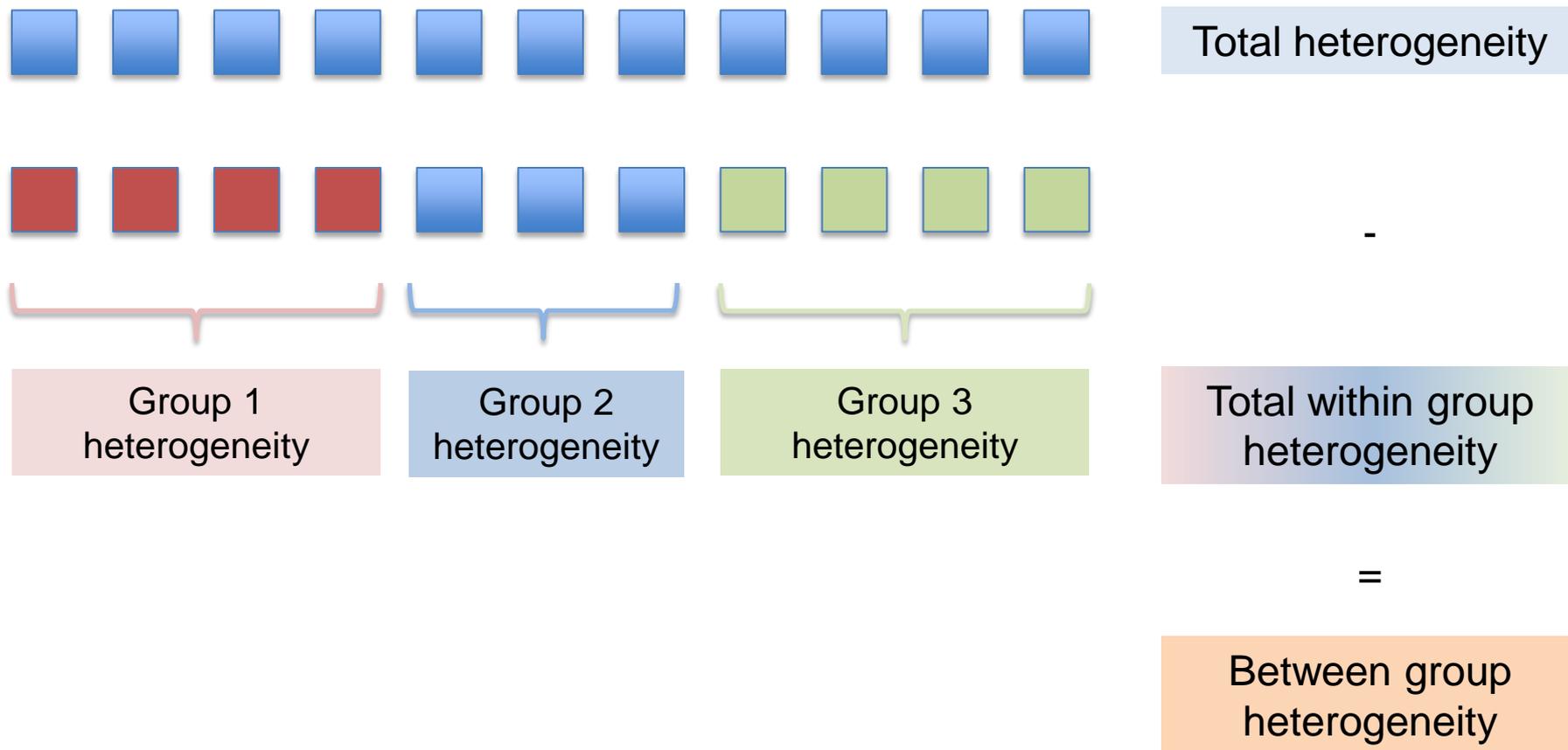
- Tau estimation

Approaches to meta-analysis

- If you expect studies to have roughly the same result, weight according to inverse variance
  - Fixed effects meta-analysis ($1/sd^2$)

- If you expect studies to have varying results, blunt FE weighting according to the extent of differences
  - Random effects meta-analysis ($1/(sd^2 + tau^2)$)

- Explore differences between studies by exploring variablity
  - Overall
  - Within studies with shared characteristics
  - Between groups of studies with different characteristics

Partitioning heterogeneity

Total heterogeneity

Group 1 heterogeneity

Group 2 heterogeneity

Group 3 heterogeneity

-

Total within group heterogeneity

=

Between group heterogeneity

Perils of testing multiple, non prespecified hypotheses

- International Study of Infarct Survival -2
  - Aspirin improves outcome in myocardial infarction

  BUT

  - non significant worsening of outcome for patients born under Gemini or Libra
  - What if it was patients with migraine?

Perils of testing multiple, non prespecified hypotheses

Odds ratios for hospitalisation for 5.3m residents of Ontario by sign of birth…

Sign	Diagnosis	Odds Ratio	p
Scorpio	Lymphoid leukemia	1.8	0.04
Scorpio	Abscess of anal and rectal region	1.57	0.01
Libra	Subarachnoid hemorrhage	1.44	0.04
Aries	Intestinal infections due to other organisms	1.41	0.01
Virgo	Excessive vomiting in pregnancy	1.4	0.03
…	…	…	…
Pisces	Other ischemic heart disease	1.1	0.02

Significantly increased odds of admission for 24 of 223 most common presentations

When tested in separate validation cohort
2 of 223

When corrected for multiple testing
0 of 223

Key messages

- *In vivo* studies which do not report simple measures to avoid bias give larger estimates of treatment effects

- Most *in vivo* studies do not report simple measures to reduce bias

- Publication and selective outcome reporting biases are important and prevalent

- You cannot assume rigour, even in Journals of "impact"

- You can only find these things out by studying large numbers of studies

- Any experimental design can be subverted; what's important is knowing how to recognise when this has happened