



National Centre
for the Replacement
Refinement & Reduction
of Animals in Research

The role of systematic reviews in improving the internal validity and reporting quality of animal research

Dr Nathalie Percie du Sert

Systematic reviews in animal research: Launch of the CAMARADES-NC3Rs Systematic Review Facility (SyRF)

London, Thursday 30 March 2017

A driver to improve research practices

Systematic reviews have been instrumental in raising the standards of clinical research

Shine a light on current practices

Provide evidence of their impact on experimental results

Systematic reviews are a driver to :

- Improve internal validity
- Improve reporting quality
- Reduce publication and reporting bias

A driver to improve research practices

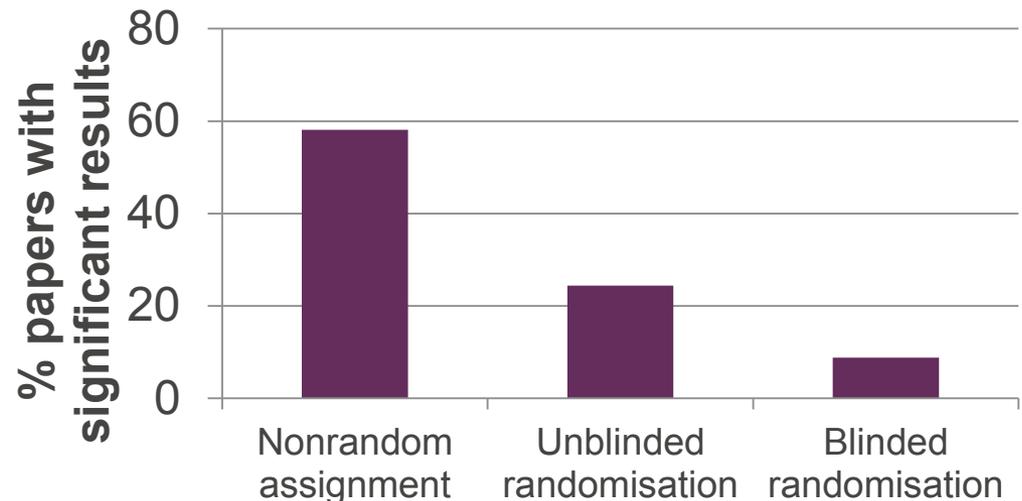
BIAS IN TREATMENT ASSIGNMENT IN CONTROLLED CLINICAL TRIALS

THOMAS C. CHALMERS, M.D., PAUL CELANO, M.D., HENRY S. SACKS, PH.D., M.D.,
AND HARRY SMITH, JR., PH.D.

Abstract Controlled clinical trials of the treatment of acute myocardial infarction offer a unique opportunity for the study of the potential influence on outcome of bias in treatment assignment. A group of 145 papers was divided into those in which the randomization process was blinded (57 papers), those in which it may have been unblinded (45 papers), and those in which the controls were selected by a nonrandom process (43 papers). At least one prognostic variable was maldistributed ($P < 0.05$) in 14.0 per cent of the blinded-randomization studies, in 26.7 per cent

of the unblinded-randomization studies, and in 58.1 per cent of the nonrandomized studies. Differences in case-fatality rates between treatment and control groups ($P < 0.05$) were found in 8.8 per cent of the blinded-randomization studies, 24.4 per cent of the unblinded-randomization studies, and 58.1 per cent of the nonrandomized studies. These data emphasize the importance of keeping those who recruit patients for clinical trials from suspecting which treatment will be assigned to the patient under consideration. (N Engl J Med 1983; 309:1358-61.)

Clinical trials of
treatment for
acute myocardial
infarction



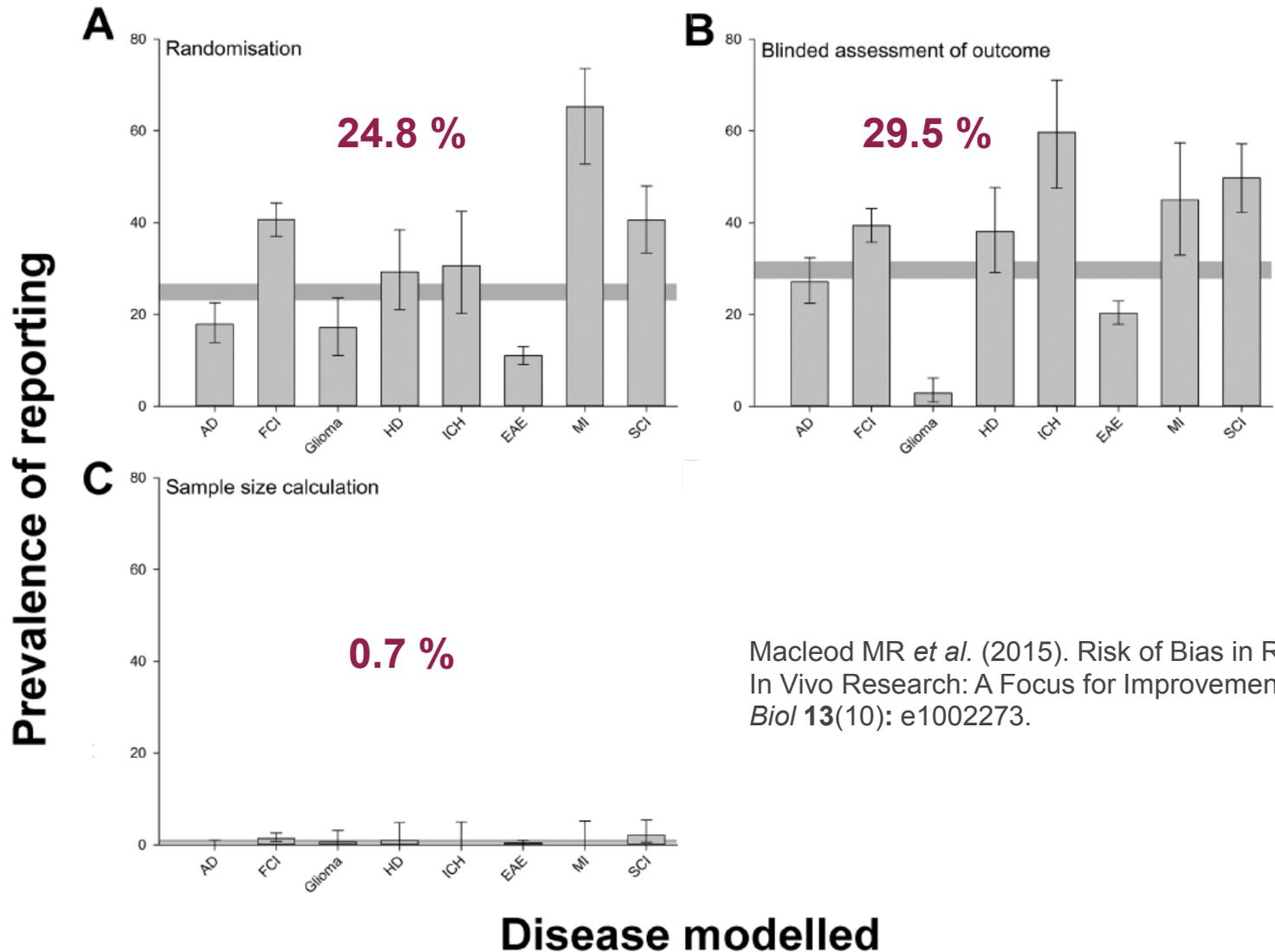
Internal validity

Risk of bias assessed as part of the systematic review

Measures used to reduce validity threats include:

- Random allocation to treatment groups
- Allocation concealment
- Blinding during outcome assessment
- Sample size determined by power calculation
- Inclusion/exclusion criteria

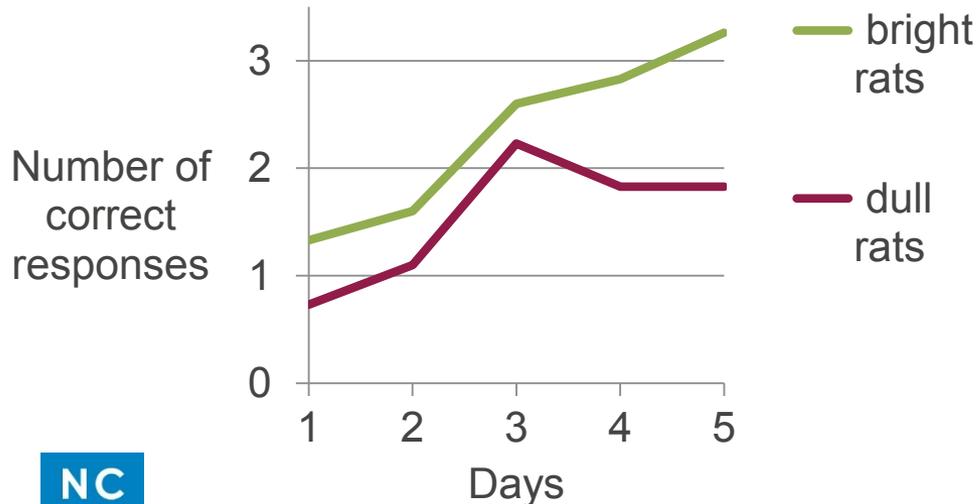
Internal validity – scale of the problem



Macleod MR *et al.* (2015). Risk of Bias in Reports of In Vivo Research: A Focus for Improvement. *PLoS Biol* 13(10): e1002273.

Internal validity – blinding

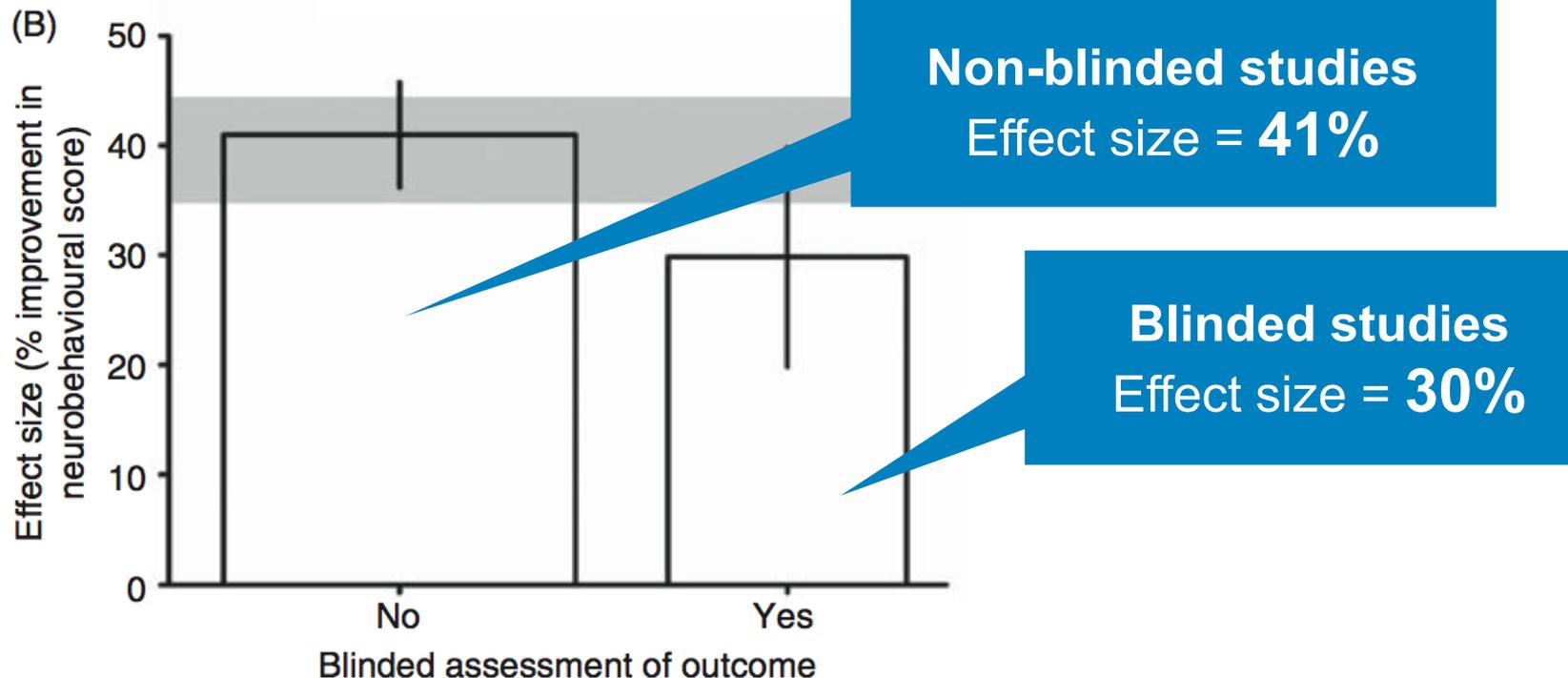
- 12 students
- Maze-bright and maze-dull rats
- Elevated T-maze, dark arm reinforced



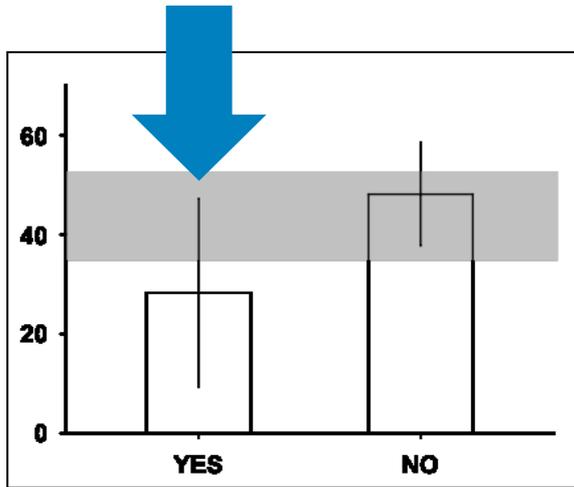
- Rats had been labelled bright or dull randomly
- Only difference was in the minds of the investigators!

Internal validity – blinding

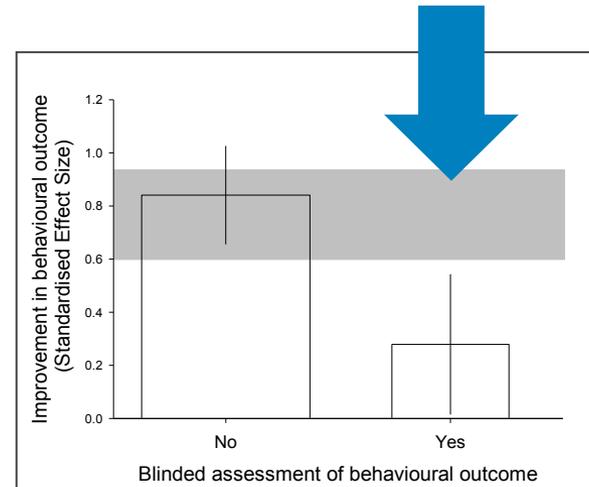
- Animal models of multiple sclerosis
- Comparison of blinded and non-blinded studies



Internal validity – blinding

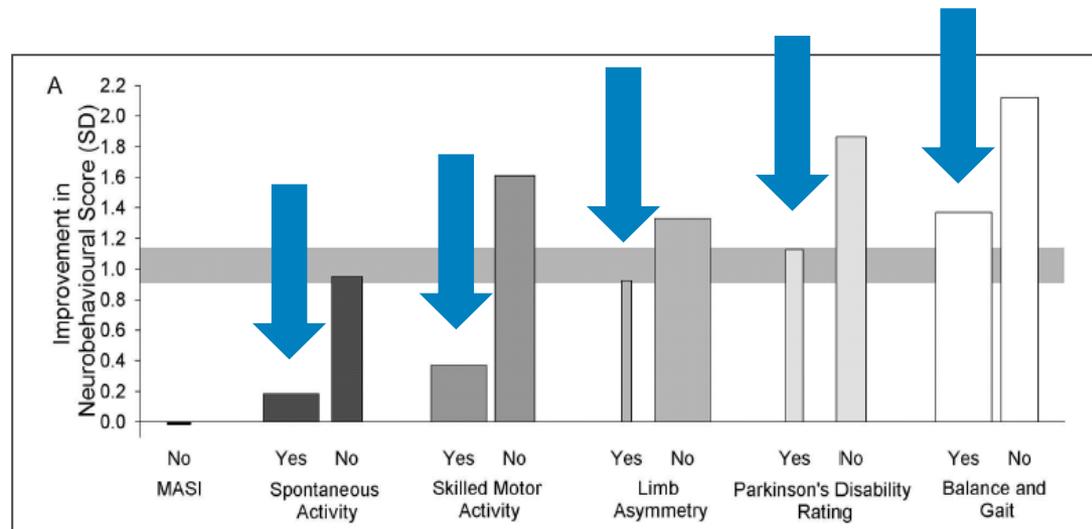


Stroke



Alzheimer's disease

All data from:



Parkinson's disease

Internal validity – randomisation

Method is important – haphazard is not random

Use a validated procedure (e.g. computer generated, throw a dice, flip a coin)

Randomisation is crucial for two reasons:

1. Minimise selection bias

e.g. haphazard selection may result in slowest mice allocated to the same group

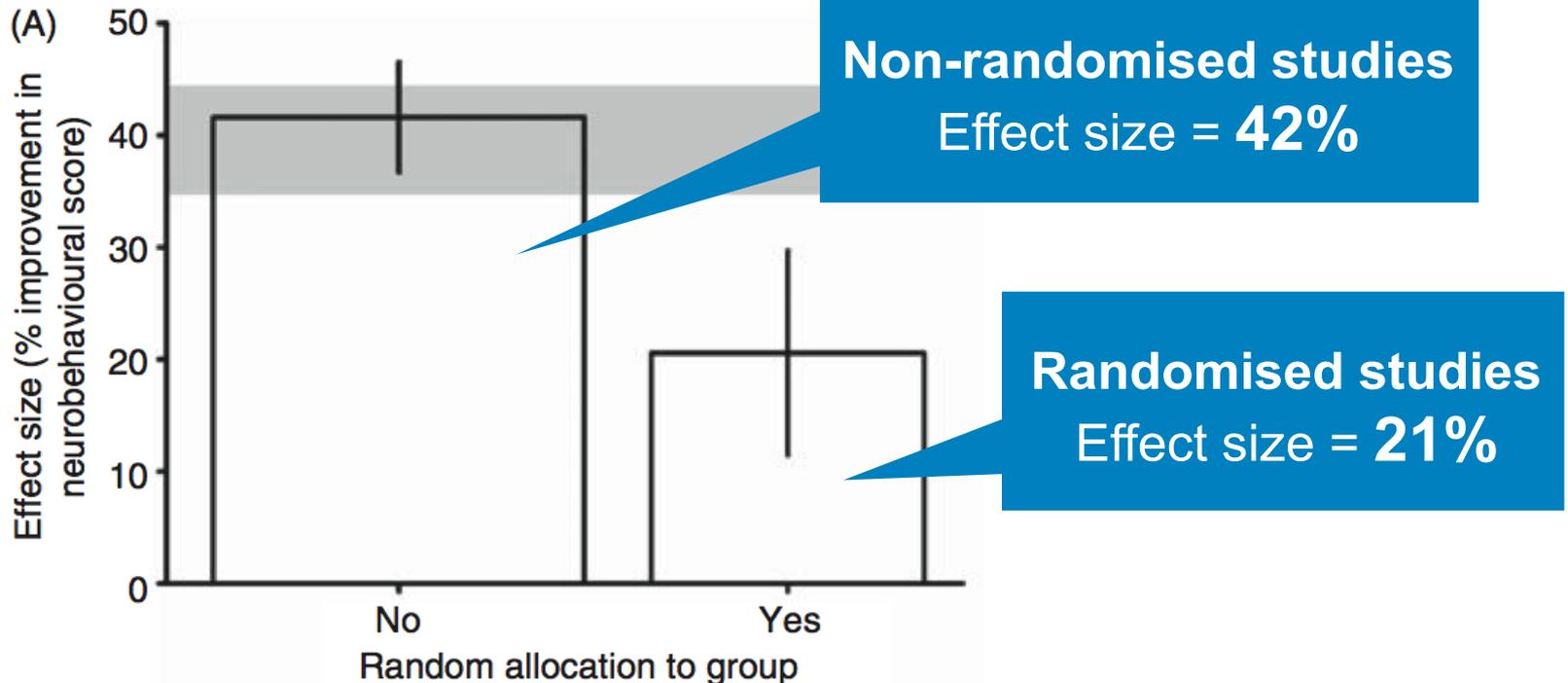
2. Key assumption of the statistical analysis

Different groups should be drawn from the same background population using random sampling



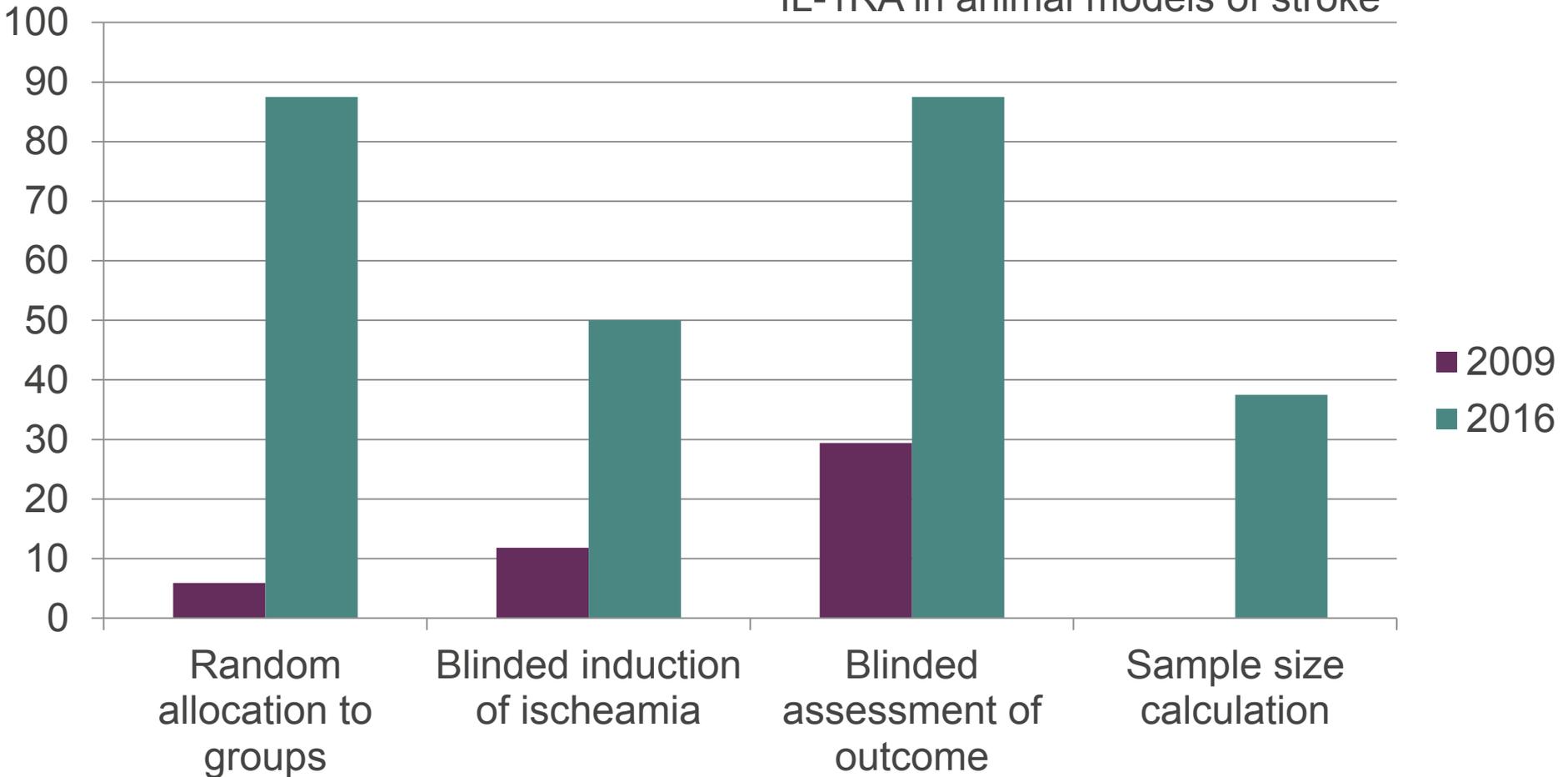
Internal validity – randomisation

- Animal models of multiple sclerosis
- Comparison of randomised and non-randomised studies



Internal validity

IL-1RA in animal models of stroke

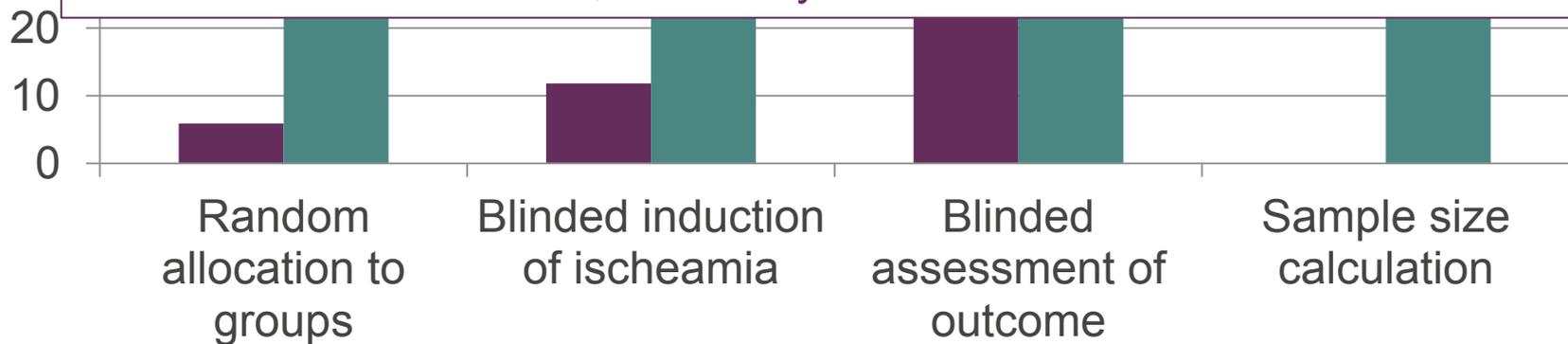


Internal validity

IL-1RA in animal models of stroke

“The 2009 systematic review highlighted areas of weakness with respect the lack of reporting on certain aspects of experimental design. While we did not necessarily agree with all recommendations and also felt that not-reported did not mean not done we did take on board that future studies did need to more fully report details of experimental design. This change is reflected in the positive outcome of the follow-up 2016 systematic review”

--- Professor Stuart Allan, University of Manchester



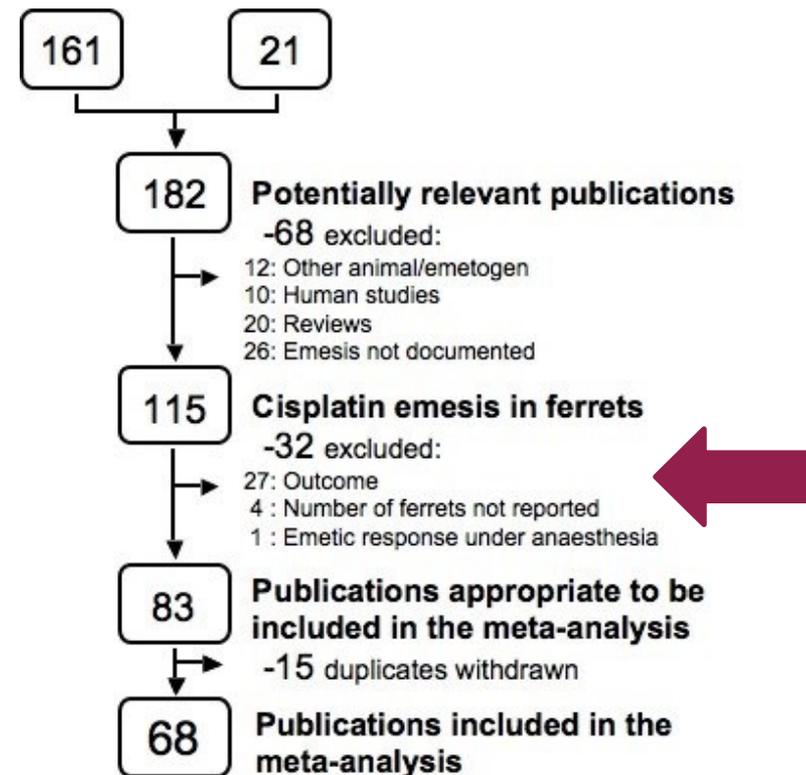
Reporting quality



Studies excluded based on:

- Outcome:
 - Not defined
 - Not consistent between studies
 - Not clinically relevant
- Number of animals not reported

In included studies, sources of heterogeneity couldn't be investigated

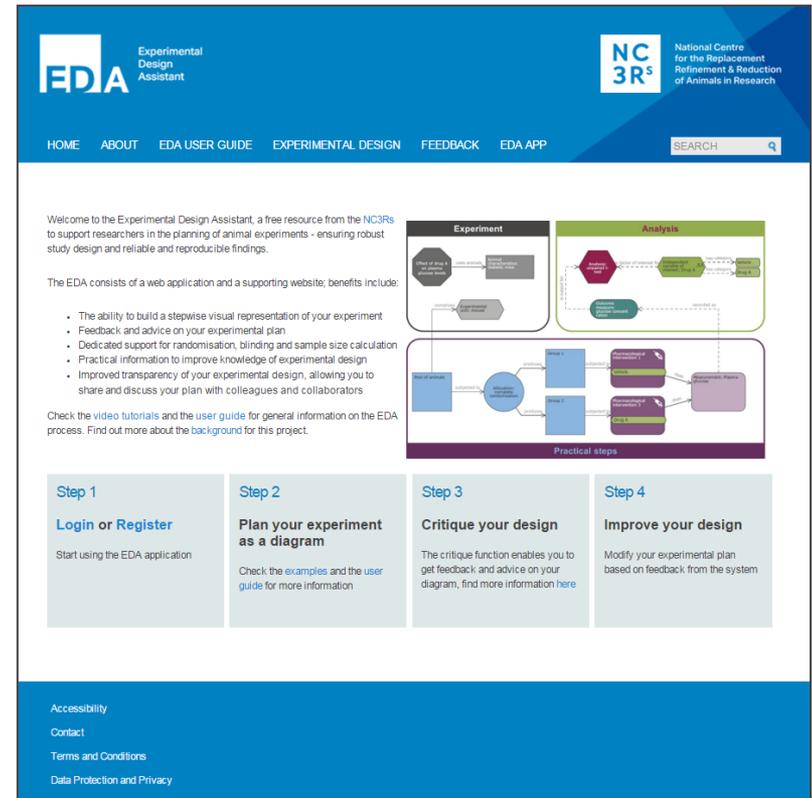


Improving internal validity and reporting

The Experimental Design Assistant

Features include:

- EDA diagram
- Critical feedback on the experimental plan
- Statistical analysis suggestions
- Sample size calculation
- Randomisation sequence generation
- Support for allocation concealment and blinding
- Web-based resources



The screenshot shows the homepage of the Experimental Design Assistant (EDA) website. The header includes the EDA logo, the text 'Experimental Design Assistant', and the NC3Rs logo with the text 'National Centre for the Replacement, Refinement & Reduction of Animals in Research'. A navigation menu contains links for HOME, ABOUT, EDA USER GUIDE, EXPERIMENTAL DESIGN, FEEDBACK, and EDA APP. A search bar is located on the right side of the header.

The main content area features a welcome message: 'Welcome to the Experimental Design Assistant, a free resource from the NC3Rs to support researchers in the planning of animal experiments - ensuring robust study design and reliable and reproducible findings.' Below this, it states 'The EDA consists of a web application and a supporting website; benefits include:' followed by a bulleted list:

- The ability to build a stepwise visual representation of your experiment
- Feedback and advice on your experimental plan
- Dedicated support for randomisation, blinding and sample size calculation
- Practical information to improve knowledge of experimental design
- Improved transparency of your experimental design, allowing you to share and discuss your plan with colleagues and collaborators

There are three diagrams illustrating the EDA process: 'Experiment' (a flowchart showing the sequence of events), 'Analysis' (a flowchart showing the analysis process), and 'Practical steps' (a flowchart showing the practical steps of the experiment). Below the diagrams, there are four steps outlined in a grid:

Step 1	Step 2	Step 3	Step 4
Login or Register	Plan your experiment as a diagram	Critique your design	Improve your design
Start using the EDA application	Check the examples and the user guide for more information	The critique function enables you to get feedback and advice on your diagram, find more information here	Modify your experimental plan based on feedback from the system

At the bottom of the page, there are links for Accessibility, Contact, Terms and Conditions, and Data Protection and Privacy.

Improving internal validity and reporting

The ARRIVE guidelines

The ARRIVE guidelines were developed to improve the reporting of biomedical research using animals.

- Checklist of 20 items, containing key information necessary to describe a study comprehensively and transparently.
- Consensus between:
 - Scientists
 - Statisticians
 - Journal editors
 - Research funders
- Used to ensure transparent and comprehensive reporting

NC 3R^s National Centre for the Replacement, Refinement and Reduction of Animals in Research

The ARRIVE Guidelines
Animal Research: Reporting of *In Vivo* Experiments

The ARRIVE (Animal Research: Reporting of *In Vivo* Experimental) guidelines were developed as part of an NC3Rs initiative to improve the design, analysis and reporting of research using animals – maximising information published and minimising unnecessary animals. The guidelines were published in the online journal *PLoS Biology* in June 2010 and are currently endorsed by scientific journals, major funding bodies and learned societies.

The guidelines are intended to:

- Improve reporting of research using animals.
- Guide authors as to the essential information that should be included in their manuscripts.

 The guidelines are NOT intended for:

- Primary authors, ethics committees, or editorial boards to be applied rigidly to items in their checklist. Some of the items are intended to be used as a guide rather than a checklist.

 What kind of research areas do the guidelines apply to?

- The guidelines will be most appropriate for experimental studies, either basic or translational, involving experimental animals or human groups of experimental animals or human subjects.

 How might these guidelines be used?

- The guidelines provide a checklist for those preparing a manuscript for peer review or for publication.

 Acknowledgements

- The NC3Rs gratefully acknowledge the expertise and advice that all the individuals from the groups listed below have provided in developing the guidelines. We would particularly like to thank:

ITEM	RECOMMENDATIONS	Checklist not mandatory	9	Provide a protocol
Title	Provide an accurate and concise description of the content of the article			
Abstract	2 Provide an accurate summary of the background, reason for diagnosis, including the scientific objectives and where appropriate, the study's experimental design and main findings of the study			
INTRODUCTION	3			
Background	1 Provide a brief scientific background including relevant references to provide context and rationale for the experimental design and rationale for the study, and explain the experimental approach to be used			
Objectives	4 Identify scientific hypothesis and any secondary objectives of the study or scientific objectives			
METHODS	5			
General approach	6 Indicate the nature of the whole-animal procedures, relevant outcomes to be measured, and where appropriate, the study's experimental design to be used in each animal, human or human group			
Study design	7 For each experimental, government-funded or funded design including: <ul style="list-style-type: none"> a. The number of experimental and control groups b. The study design (randomised, parallel or sequential) and other designing details for both (e.g. randomised or blinded) and other assessing results (e.g. blinding, randomisation, and other details) c. The experimental unit (e.g. single animal, group or cage of animals) d. Allocation sequence or flow chart (where used) to ensure the complete study design is described 			
Experimental animals	8 For each experimental, government-funded or funded design including: <ul style="list-style-type: none"> a. The number of animals used in each experimental group, including details of any animals that were excluded or culled b. The experimental unit (e.g. single animal, group or cage of animals) c. Allocation sequence or flow chart (where used) to ensure the complete study design is described 			
Operational methods	9 For each experimental, government-funded or funded design including: <ul style="list-style-type: none"> a. The number of animals used in each experimental group, including details of any animals that were excluded or culled b. The experimental unit (e.g. single animal, group or cage of animals) c. Allocation sequence or flow chart (where used) to ensure the complete study design is described 			
Statistical analysis	10 For each experimental, government-funded or funded design including: <ul style="list-style-type: none"> a. The number of animals used in each experimental group, including details of any animals that were excluded or culled b. The experimental unit (e.g. single animal, group or cage of animals) c. Allocation sequence or flow chart (where used) to ensure the complete study design is described 			
Results	11 For each experimental, government-funded or funded design including: <ul style="list-style-type: none"> a. The number of animals used in each experimental group, including details of any animals that were excluded or culled b. The experimental unit (e.g. single animal, group or cage of animals) c. Allocation sequence or flow chart (where used) to ensure the complete study design is described 			
Discussion	12 For each experimental, government-funded or funded design including: <ul style="list-style-type: none"> a. The number of animals used in each experimental group, including details of any animals that were excluded or culled b. The experimental unit (e.g. single animal, group or cage of animals) c. Allocation sequence or flow chart (where used) to ensure the complete study design is described 			
Conclusions	13 For each experimental, government-funded or funded design including: <ul style="list-style-type: none"> a. The number of animals used in each experimental group, including details of any animals that were excluded or culled b. The experimental unit (e.g. single animal, group or cage of animals) c. Allocation sequence or flow chart (where used) to ensure the complete study design is described 			
References	14 For each experimental, government-funded or funded design including: <ul style="list-style-type: none"> a. The number of animals used in each experimental group, including details of any animals that were excluded or culled b. The experimental unit (e.g. single animal, group or cage of animals) c. Allocation sequence or flow chart (where used) to ensure the complete study design is described 			
Acknowledgements	15 For each experimental, government-funded or funded design including: <ul style="list-style-type: none"> a. The number of animals used in each experimental group, including details of any animals that were excluded or culled b. The experimental unit (e.g. single animal, group or cage of animals) c. Allocation sequence or flow chart (where used) to ensure the complete study design is described 			
Supplementary information	16 For each experimental, government-funded or funded design including: <ul style="list-style-type: none"> a. The number of animals used in each experimental group, including details of any animals that were excluded or culled b. The experimental unit (e.g. single animal, group or cage of animals) c. Allocation sequence or flow chart (where used) to ensure the complete study design is described 			
Declarations of interest	17 For each experimental, government-funded or funded design including: <ul style="list-style-type: none"> a. The number of animals used in each experimental group, including details of any animals that were excluded or culled b. The experimental unit (e.g. single animal, group or cage of animals) c. Allocation sequence or flow chart (where used) to ensure the complete study design is described 			
Supplementary information	18 For each experimental, government-funded or funded design including: <ul style="list-style-type: none"> a. The number of animals used in each experimental group, including details of any animals that were excluded or culled b. The experimental unit (e.g. single animal, group or cage of animals) c. Allocation sequence or flow chart (where used) to ensure the complete study design is described 			
References	19 For each experimental, government-funded or funded design including: <ul style="list-style-type: none"> a. The number of animals used in each experimental group, including details of any animals that were excluded or culled b. The experimental unit (e.g. single animal, group or cage of animals) c. Allocation sequence or flow chart (where used) to ensure the complete study design is described 			
Conclusions	20 For each experimental, government-funded or funded design including: <ul style="list-style-type: none"> a. The number of animals used in each experimental group, including details of any animals that were excluded or culled b. The experimental unit (e.g. single animal, group or cage of animals) c. Allocation sequence or flow chart (where used) to ensure the complete study design is described 			

Improving internal validity and reporting

The ARRIVE guidelines

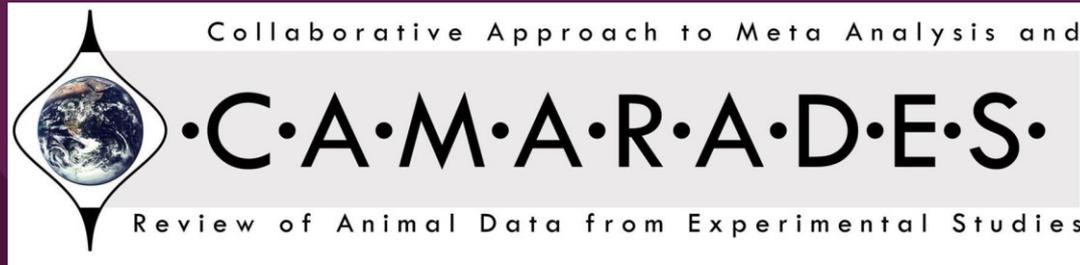
The guidelines include:

- Information which relates to internal validity
- Information which would allow a study to be repeated
- Information about the context and scientific relevance of the study

Using the guidelines ensures that a study contains enough information:

- to be appropriately identified in search strategies
- to assess the risk of bias
- to investigate sources of heterogeneity

Acknowledgements:



Further information:

www.nc3rs.org.uk

@NC3Rs

nathalie.perciedusert@nc3rs.org.uk

NC
3R^s

