Practical issues in preclinical study design

part 1

Anton Bespalov

Partnership for Assessment and Accreditation of Scientific Practice

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PAASP

What kind of research do you do?

- Preclinical?
- Brain injury?
- In vivo?
- Between-subject study designs?

Why do you do experiments?

- ~~I have to publish …~~
- ~~Part of my job desciption~~
- ~~Just for the fun of it~~

Why do you do experiments?

- Others will recognize value in what I do, will use and benefit from the data that I generate

Exploratory vs confirmatory research

- We need both exploratory and confirmatory research
- Decision-making requires research where various measures are taken to control selection, detection, attrition, performance, and reporting bias

	Exploratory (Discovery)	Confirmatory
Hypothesis	+	+++
Establish pathophysiology	+++	+
Sequence and details of experiments established at onset	+	+++
Defined primary end point	–	++
Sample size calculation	+	+++
Blinding	+++	+++
Randomization	+++	+++
External validity (aging, comorbidities, etc)	–	++
Predefined inclusion/exclusion criteria	++	+++
Test statistics	+	+++
Preregistration	–	++
High sensitivity (high type I error rate, low type II error rate): find what might work	+++	+
High specificity (low type I error rate, high type II error rate): weed out false-positives	+	+++

Dirnagl (2016) Stroke 47:2148

Exploratory vs confirmatory research

- No commonly agreed definitions
  - Is there something else beyond ”exploratory” and “confirmatory”?
  - Where does “exploration” end and “confirmation” start?

Major discovery can trigger a Phase II study

ApoE-Directed Therapeutics Rapidly Clear β-Amyloid and Reverse Deficits in AD Mouse Models

Paige E. Cramer,[1] John R. Cirrito,[2] Daniel W. Wesson,[1,3] C. Y. Daniel Lee,[1] J. Colleen Karlo,[1] Adriana E. Zinn,[1] Brad T. Casali,[1] Jessica L. Restivo,[2] Whitney D. Goebel,[2] Michael J. James,[4] Kurt R. Brunden,[4] Donald A. Wilson,[3] Gary E. Landreth[1]*

Alzheimer's disease (AD) is associated with impaired clearance of β-amyloid (Aβ) from the brain, a process normally facilitated by apolipoprotein E (apoE). ApoE expression is transcriptionally induced through the action of the nuclear receptors peroxisome proliferator–activated receptor gamma and liver X receptors in coordination with retinoid X receptors (RXRs). Oral administration of the RXR agonist bexarotene to a mouse model of AD resulted in enhanced clearance of soluble Aβ within hours in an apoE-dependent manner. Aβ plaque area was reduced more than 50% within just 72 hours. Furthermore, bexarotene stimulated the rapid reversal of cognitive, social, and olfactory deficits and improved neural circuit function. Thus, RXR activation stimulates physiological Aβ clearance mechanisms, resulting in the rapid reversal of a broad range of Aβ-induced deficits.

SCIENCE VOL 335 23 MARCH 2012

… before it is properly confirmed

Comment on "ApoE-Directed Therapeutics Rapidly Clear β-Amyloid and Reverse Deficits in AD Mouse Models"

Ina Tesseur,[1,2]* Adrian C. Lo,[3]* Anouk Roberfroid,[1,2] Sofie Dietvorst,[1] Bianca Van Broeck,[4] Marianne Borgers,[4] Harrie Gijsen,[4] Diederik Moechars,[4] Marc Mercken,[4] John Kemp,[4] Rudi D'Hooge,[3] Bart De Strooper[1,2]†

Cramer *et al.* (Reports, 23 March 2012, p. 1503; published online 9 February 2012) tested bexarotene as a potential β-amyloid–lowering drug for Alzheimer's disease (AD). We were not able to reproduce the described effects in several animal models. Drug formulation appears very critical. Our data call for extreme caution when considering this compound for use in AD patients.

Comment on "ApoE-Therapeutics Rapidl… β-Amyloid and Reve… AD Mouse Models"

Nicholas F. Fitz, Andrea A. Cronican, Iliya Lefterov,* Radosveta Koldamova*

Cramer *et al.* (Reports, 23 March 2012, p. 1503; published online 9 February 2012) demonstrated in a mouse model for Alzheimer's disease (AD) that treatment of APP/PS1ΔE9 mice with bexarotene decreased Aβ pathology and ameliorated memory deficits. We confirm the reversal of memory deficits in APP/PS1ΔE9 mice expressing human APOE3 or APOE4 to the levels of their nontransgenic controls and the significant decrease of interstitial fluid Aβ, but not the effects on amyloid deposition.

…t on "ApoE-Directed Therapeutics …Clear β-Amyloid and Reverse …in AD Mouse Models"

Ashleigh R. Price,* Guilian Xu,* Zoe B. Siemienski, Lisa A. Smithson, David R. Borchelt, Todd E. Golde, Kevin M. Felsenstein†

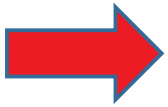
Cramer *et al.* (Reports, 23 March 2012, p. 1503; published online 9 February 2012) demonstrates short-term bexarotene treatment clearing preexisting β-amyloid deposits from the brains of APP/PS1ΔE9 mice with low amyloid burden, providing a rationale for repurposing this anticancer agent as an Alzheimer's disease (AD) therapeutic. Using a nearly identical treatment regimen, we were unable to detect any evidence of drug efficacy despite demonstration of target engagement.

SCIENCE VOL 340 24 MAY 2013

NATURE MEDICINE

PD-1 immune checkpoint blockade reduces pathology and improves memory in mouse models of Alzheimer's disease

Kuti Baruch[1], Aleksandra Deczkowska[1], Neta Rosenzweig[1], Afroditi Tsitsou-Kampeli[1], Alaa Mohammad Sharif[1], Orit Matcovitch-Natan[1,2], Alexander Kertser[1], Eyal David[2], Ido Amit[2] & Michal Schwartz[1]



Received: 22 September 2017 | Revised: 20 October 2017 | Accepted: 24 October 2017
DOI: 10.1002/glia.23260

GLIA WILEY

RESEARCH ARTICLE

Systemic immune-checkpoint blockade with anti-PD1 antibodies does not alter cerebral amyloid-β burden in several amyloid transgenic mouse models

Martine Latta-Mahieu[1] | Bradford Elmer[2] | Alexis Bretteville[3] | Yaming Wang[4] | Mati Lopez-Grancha[1] | Philippe Goniot[1] | Nicolas Moindrot[1] | Paul Ferrari[5] | Véronique Blanc[5] | Nathalie Schussler[1] | Emmanuel Brault[1] | Valérie Roudières[1] | Véronique Blanchard[1] | Zhi-Yong Yang[2] | Pascal Barneoud[1] | Philippe Bertrand[1] | Bart Roucourt[6] | Sofie Carmans[6] | Astrid Bottelbergs[3] | Liesbeth Mertens[3] | Cindy Wintmolders[3] | Peter Larsen[3] | Caroline Hersley[4] | Tyler McGathey[4] | Margaret M. Racke[4] | Ling Liu[4] | Jirong Lu[4] | Michael J. O'Neill[4] | David R. Riddell[4] | Andreas Ebneth[3] | Gary J. Nabel[2] | Laurent Pradier[1]

… inhibition of PD1 checkpoint signaling by itself is not sufficient to reduce amyloid pathology and that additional factors might have contributed to previously published results (Baruch et al., (2016): Nature Medicine, 22:135–137). Until such factors are elucidated, animal model ***data do not support further evaluation*** *of PD1 checkpoint inhibition as a therapeutic modality for Alzheimer's disease.*

What is confirmatory research?

- Confirmatory ≠ “Successful” follow-up
  - Two independent poorly designed studies may have as little value as a single poorly designed study
- Confirmatory research:
  - Engages extra “defense” to protect against risks of bias
  - Builds confidence
  - Enables decisions
- You decide:
  - How much confidence is needed for which decisions
  - How much protection, when and where to apply

Measures that bring confidence

- Pre-specified hypothesis & data analysis, sample size and power adequate to test the hypothesis, randomization, blinding, pre-defined inclusion / exclusion criteria, deviations from protocol controlled and reported, and so on

From the pre-workshop survey

1. Do you find it difficult to apply randomization in your studies?		
Yes	0	0%
No	8	80%
no answer	2	20%

3. Do you find it difficult to apply blinding in your studies?		
Yes	1	10%
No	7	70%
no answer	2	20%

5. Do you usually pre-specify a research hypothesis before doing a study?		
Yes	8	80%
No	0	0%
no answer	2	20%

Improving Bioscience Research Reporting: The ARRIVE Guidelines for Reporting Animal Research

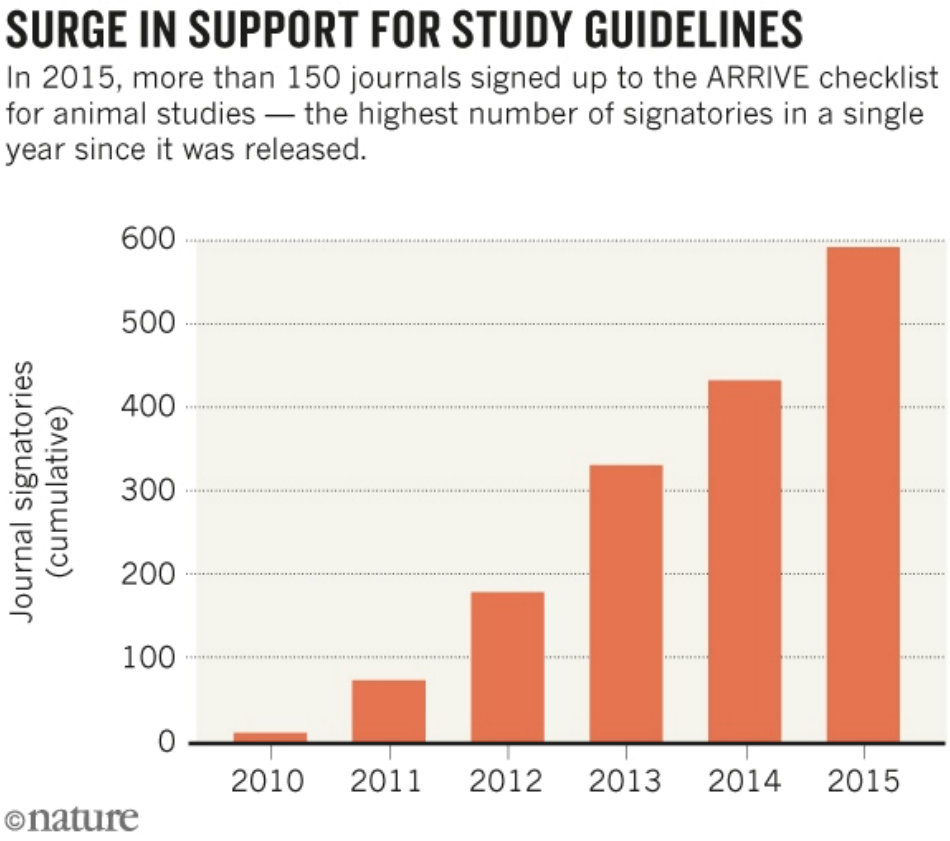
Carol Kilkenny[1]*, William J. Browne[2], Innes C. Cuthill[3], Michael Emerson[4], Douglas G. Altman[5]

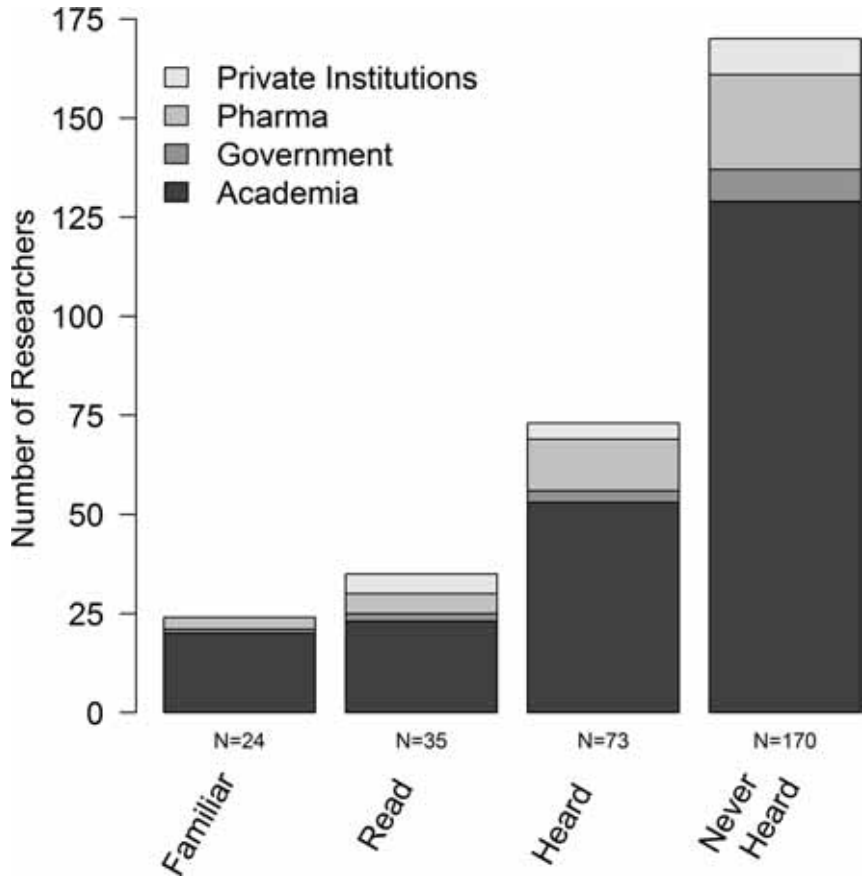
1 The National Centre for the Replacement, Refinement and Reduction of Animals in Research, London, United Kingdom, **2** School of Veterinary Science, University of Bristol, Bristol, United Kingdom, **3** School of Biological Sciences, University of Bristol, Bristol, United Kingdom, **4** National Heart and Lung Institute, Imperial College London, United Kingdom, **5** Centre for Statistics in Medicine, University of Oxford, Oxford, United Kingdom

Sample size	10	a. Specify the total number of animals used in each experiment, and the number of animals in each experimental group. b. Explain how the number of animals was arrived at. Provide details of any sample size calculation used. c. Indicate the number of independent replications of each experiment, if relevant.
Allocating animals to experimental groups	11	a. Give full details of how animals were allocated to experimental groups, including randomisation or matching if done. b. Describe the order in which the animals in the different experimental groups were treated and assessed.
Experimental outcomes	12	Clearly define the primary and secondary experimental outcomes assessed (e.g. cell death, molecular markers, behavioural changes).
Statistical methods	13	a. Provide details of the statistical methods used for each analysis. b. Specify the unit of analysis for each dataset (e.g. single animal, group of animals, single neuron). c. Describe any methods used to assess whether the data met the assumptions of the statistical approach.

Kilkenny et al (2010) PLoS Biol 8(6): e1000412

Supported but still not known?

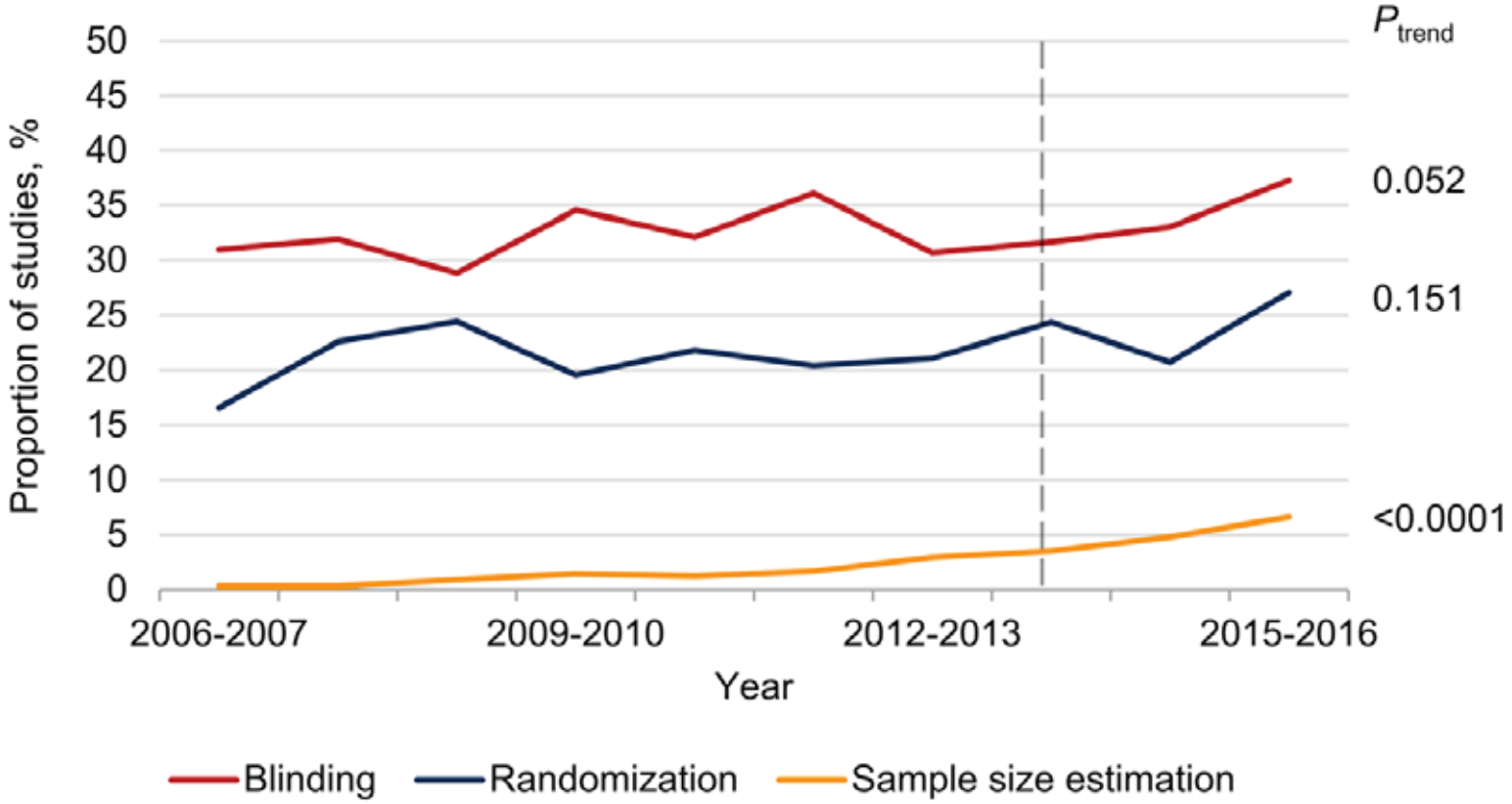




Cressey (2016) Nature

Reichlin et al (2016) PLoS ONE 11(12): e0165999

Not known and not followed?



Ramirez et al (2017) Circ Res 120:1916-1926

Reporting Checklist For Life Sciences Articles

This checklist is used to ensure good reporting standards and to improve the reproducibility of published results. For more information, please read Reporting Life Sciences Research.

is published

This checklist ~~will not be published~~. Please ensure that the answers to the following questions are reported in the manuscript itself. We encourage you to include a specific subsection in the Methods section for statistics, reagents and animal models. Below, provide the page number or section and paragraph number (e.g. “Page 5” or “Methods, ‘reagents’ subsection, paragraph 2”).

Animal Models

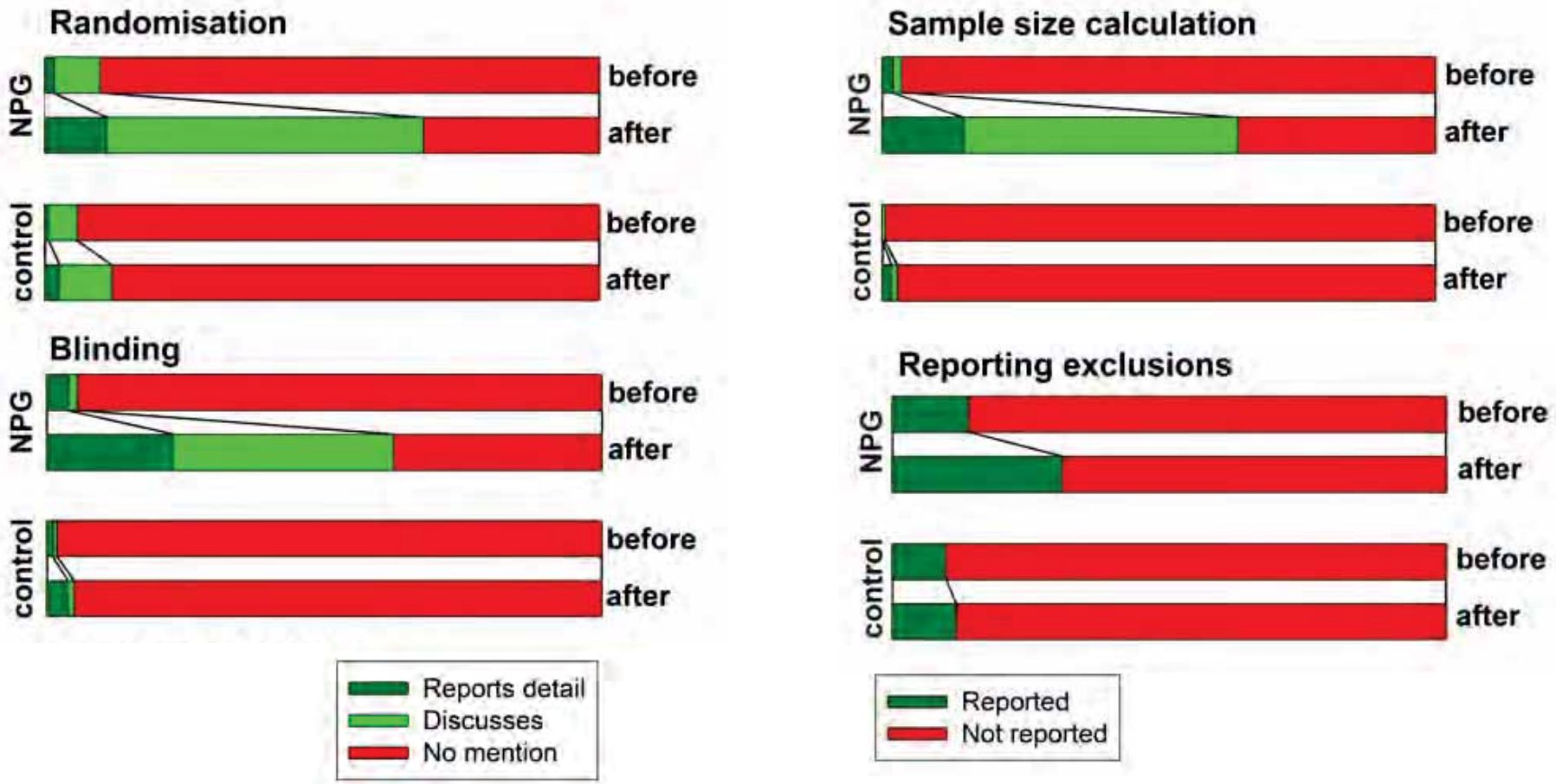
Reported in section/paragraph or page #:

8. Report species, strain, sex and age of animals

9. For experiments involving live vertebrates, include a statement of compliance with ethical regulations and identify the committee(s) approving the experiments.

10. We recommend consulting the ARRIVE guidelines (PLoS Biol. 8(6), e1000412,2010) to ensure that other relevant aspects of animal studies are adequately reported.

Do the checklists have an impact?



Macleod, The NPQIP Collaborative group (2017) BioRxiv

What is our goal?

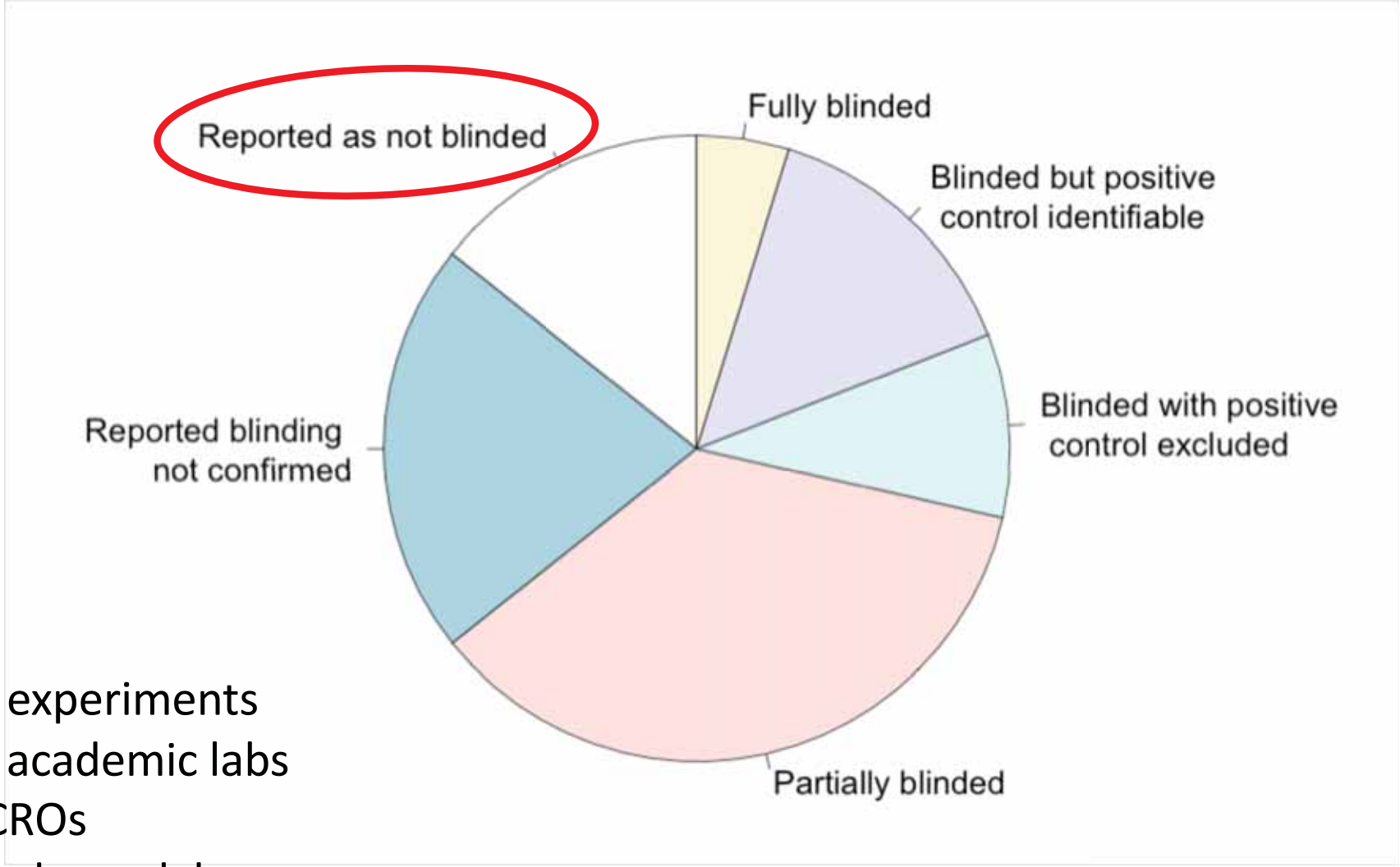
"Asking for more detailed information at the planning stage of the research might also reduce the danger of normative responses, whereby scientists simply satisfy the guidelines (e.g., ARRIVE) at a time when it is too late to take corrective actions on experimental conduct."

Vogt et al (2016) PLoS Biol 14(12): e2000598

Example (paper published):

Sample size Describe how sample size was determined.	The sample size was determined based on preliminary results or similar experiments carried-out in the past. Power Analysis was performed using G-power in order to estimate the number of animals required, for a signal-to-noise ratio of 1.4 and 80% to 90% power assuming a 5% significance level. This is stated in the Statistical analysis section in Methods.
Replication Describe whether the experimental findings were reliably reproduced.	The attempts at replication were successful.

Varieties of blinding in pharmacology



- 72 experiments
- 12 academic labs
- 7 CROs
- 4 industry labs

‘When I use a word,’ Humpty Dumpty said in rather a scornful tone, ‘it means just what I choose it to mean — neither more nor less.’

Lewis Carroll (1871)

Through the Looking-Glass, and What Alice Found There

Measures that bring confidence

- Pre-specified hypothesis & data analysis, sample size and power adequate to test the hypothesis, randomization, blinding, pre-defined inclusion / exclusion criteria, deviations from protocol controlled and reported, and so on

- May appear as a burden but it is typically easier to implement research rigor measures than to argue why they do not apply in your case

Practical issues in preclinical study design

part 2

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Pre-specified …

- … hypothesis
- … data analysis
- … exclusion criteria
- …

Why do we need to pre-specify?

- Rationalization („making excuses“)
  A defense mechanism in which controversial behaviors or feelings are justified and explained in a seemingly rational or logical manner to avoid the true explanation, and are made consciously tolerable - or even admirable and superior - by plausible means

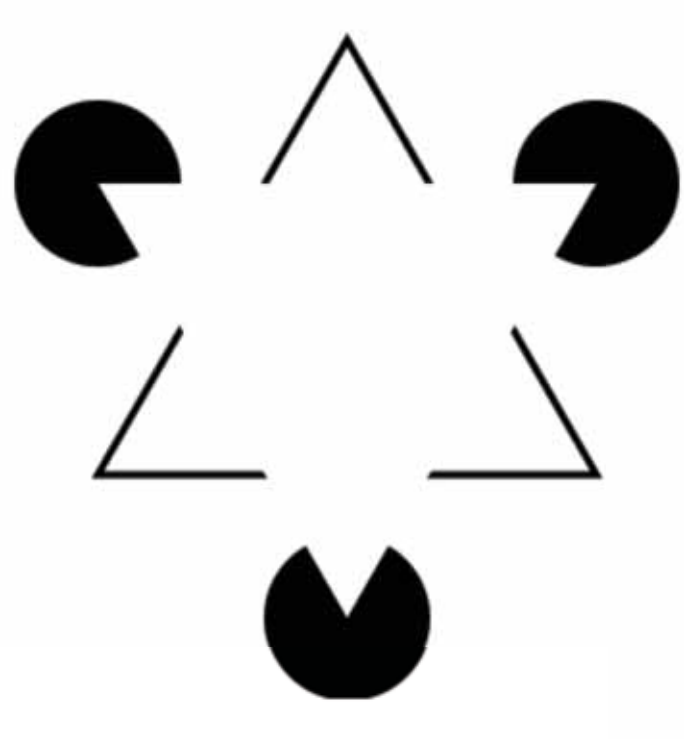
  I didn't get the job that I applied for, but I really didn't want it in the first place

Does this sound familiar?

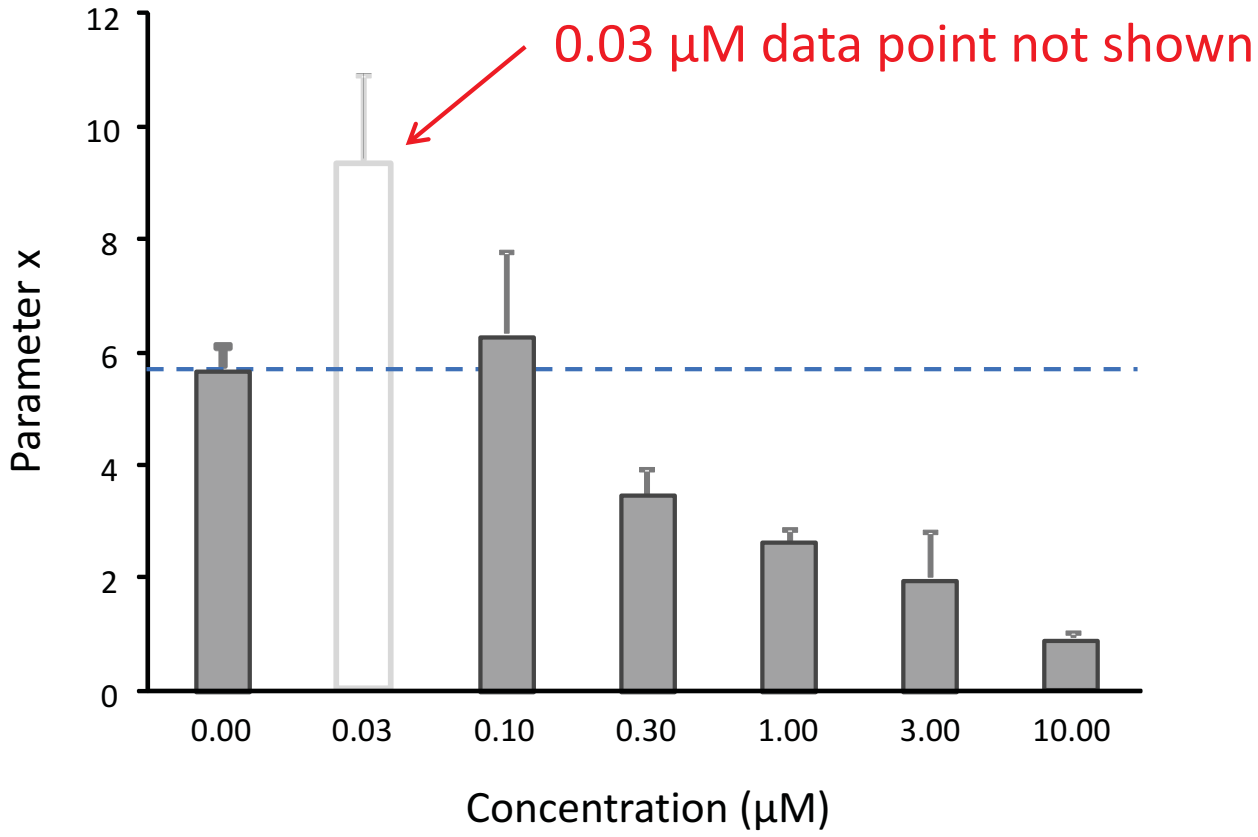
- You are running an experiment and there is a sudden noise, vibration or some other factor
- You get angry but cannot do anything other than recording in your lab journal that such event occurred at this particular time point
- When analyzing the data, you notice that one of the subjects / data points behaves strange and it is exactly when that disturbing interference occurred
- What do you do?

Why do we need to pre-specify?

- Rationalization („making excuses“)
- “Pattern completion” ability



A real-life example …



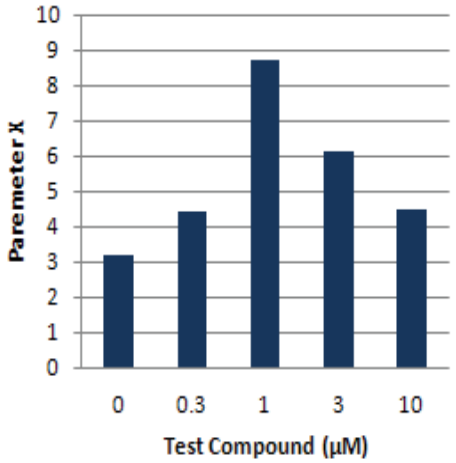
Why do we need to pre-specify?

- Rationalization („making excuses“)
- “Pattern completion” ability
- Pressure

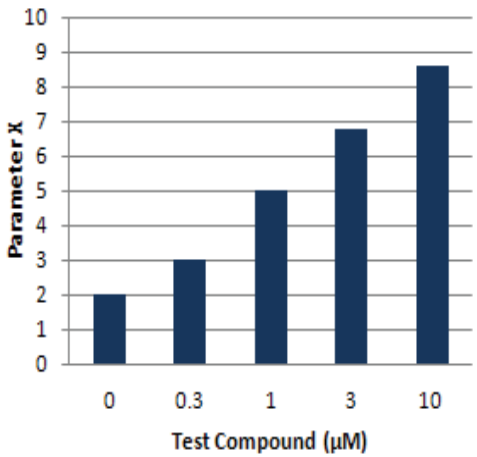
Another real-life example …

One compound was tested twice and following results were generated.
Are the reported results a complete representation of the experiments done?

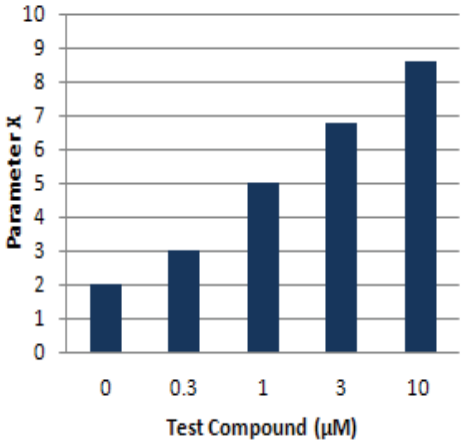
Experiment 1



Experiment 2

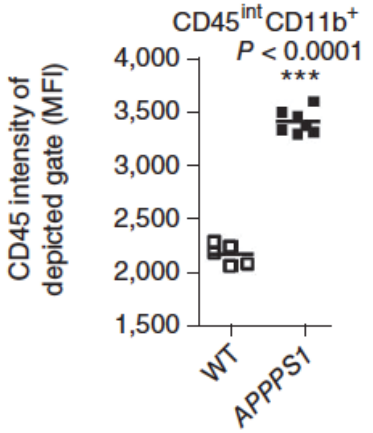


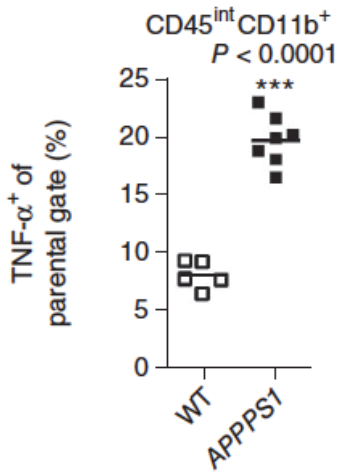
Results reported



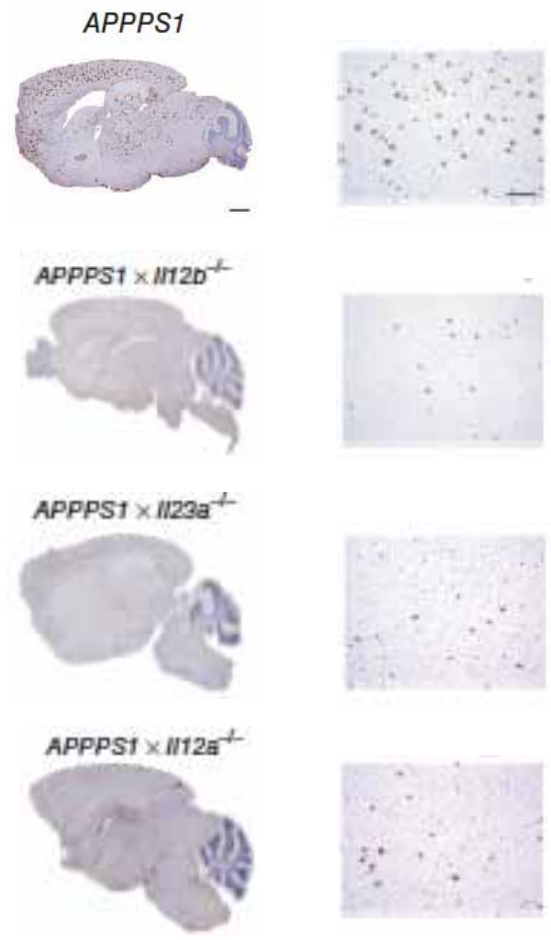
Pressure-generating hypothesis

Elevated glial cytokines in AD





Deletion of IL12/23 subunits reduces Aß plaque load



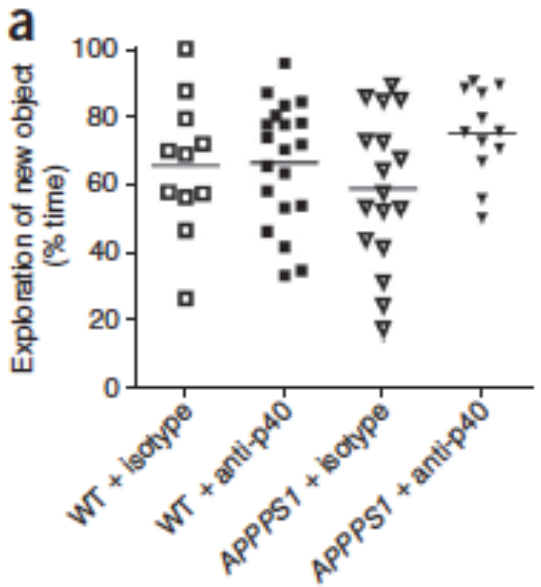
Functional outcome

ICV delivery of p40 antibody reversed cognitive deficits in aged APP/PS1 mice

vom Berg et al (2012) Nat Med 18:1812-9

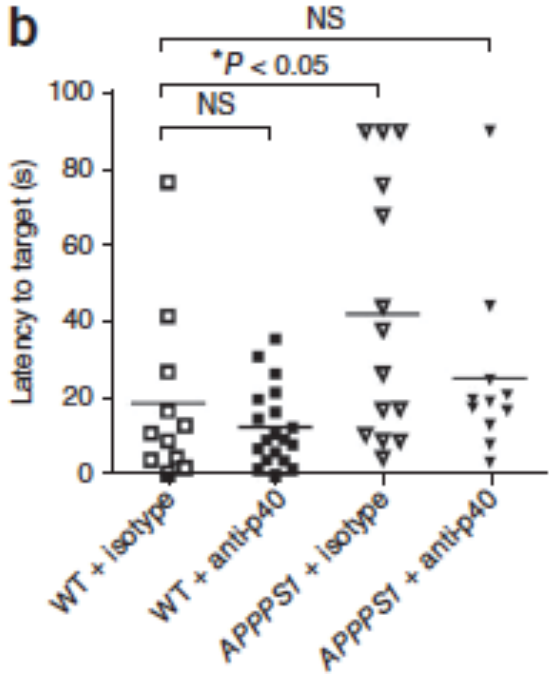
Inhibition of IL-12/IL-23 signaling reduces Alzheimer's disease–like pathology and cognitive decline

Novel object recognition



One-way ANOVA:
„P<0.05“
Post hoc: not shown

Barnes maze



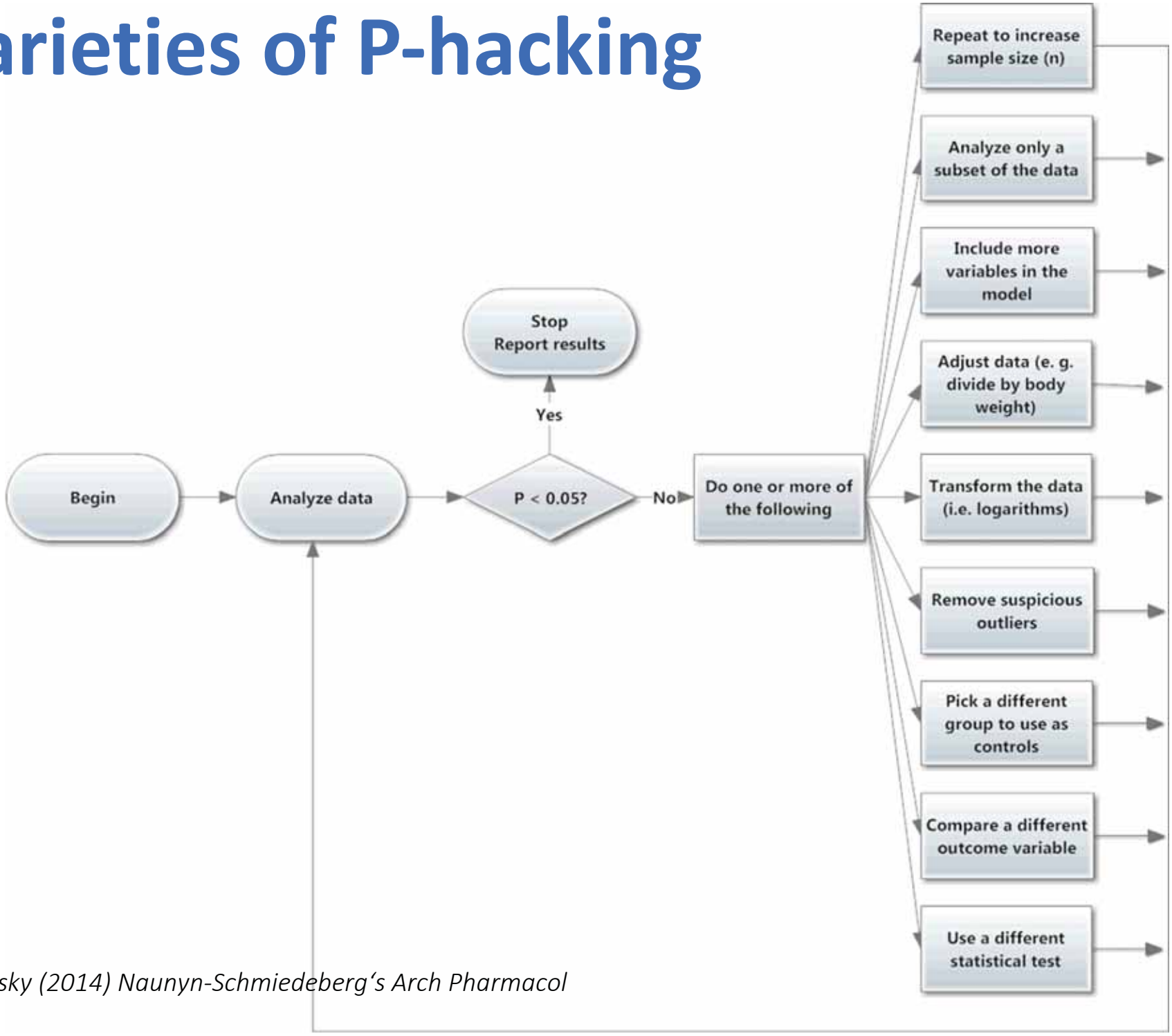
ANOVA: not shown
Post hoc: Dunnett's

Fear conditioning

Stated in the text:
„... performance in the contextual fear conditioning test did not differ between p40-antibody–treated and isotype-treated APPPS1 mice (data not shown)“

vom Berg et al (2012) Nat Med 18:1812-9

Varieties of P-hacking



Motulsky (2014) Naunyn-Schmiedeberg's Arch Pharmacol

How can we pre-specify?

- Pre-commit: Make the decision before you're in the tempting situation
  - Only take a limited amount of money with you to curtail spending
  - Have only healthy foods at home to avoid the temptation to go astray
  - It is difficult to pre-commit because normally we like to leave our options open

powerdragons.org

How can we pre-specify?

- Lab notebook
  - paper-based or electronic
- Preregistration
  - https://cos.io/prereg/
  - https://preclinicaltrials.eu
- Further ideas?

powerdragons.org

What can we pre-specify?

- Key study and analysis details **and** decisions

„Outlier“ exclusion

Included
Excluded

	rat1_Veh	rat10_Veh	rat7_Veh	rat2_10	rat9_10	rat3_30	rat5_30	rat12_30
nM	2.88	2.25	5.22	5.58 ★	3.87	3.06	2.43 ★	4.14
	2.07	3.78	2.34	2.07	4.86	1.53	9.62	6.56
	2.97	5.13	1.80	3.87	6.83	1.62	4.95	4.68
	10.79	2.16	6.74	3.06	3.06	2.34	10.88 ★	3.51
	8.45	2.88	2.52	4.50 ★	3.60	4.68	5.04	10.07

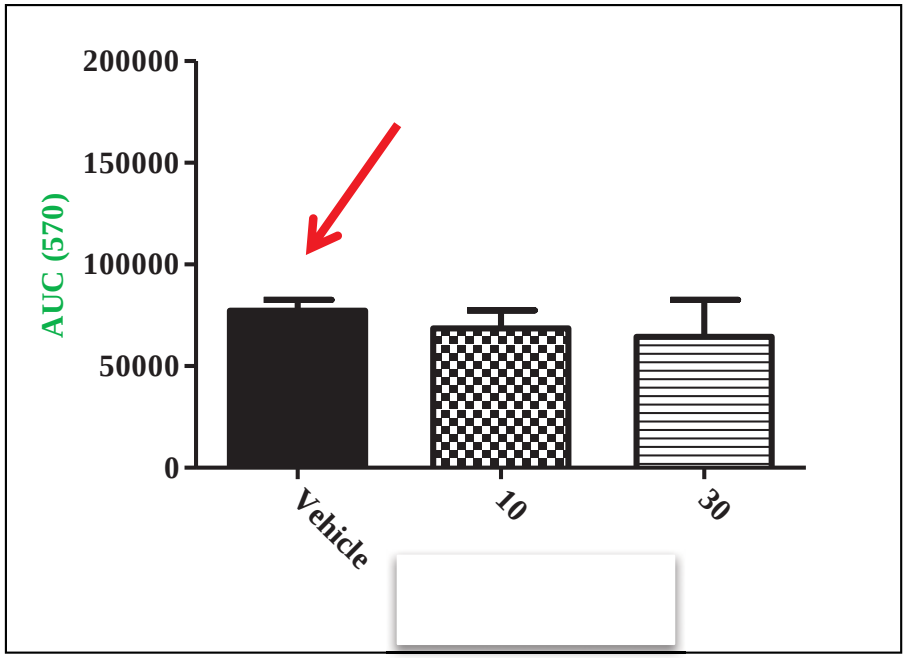
	rat4_Veh	rat9_Veh	rat7_10	rat10_10	rat12_10	rat6_30	rat11_30
nM	1.35	1.80	2.34	4.14	1.80	1.80	1.89
	0.99	3.51	2.79	3.42	0.99	1.08	0.54
	3.87	2.61	2.25	1.98	0.99 ★	0.90	1.26
	2.52 ★	1.53	3.42	2.88	0.99 ★	3.06	1.44
	1.17	1.35	2.25	2.70	2.43	2.07	5.31

★ **Why included?**

<u>No criteria</u> for exclusion of data points defined

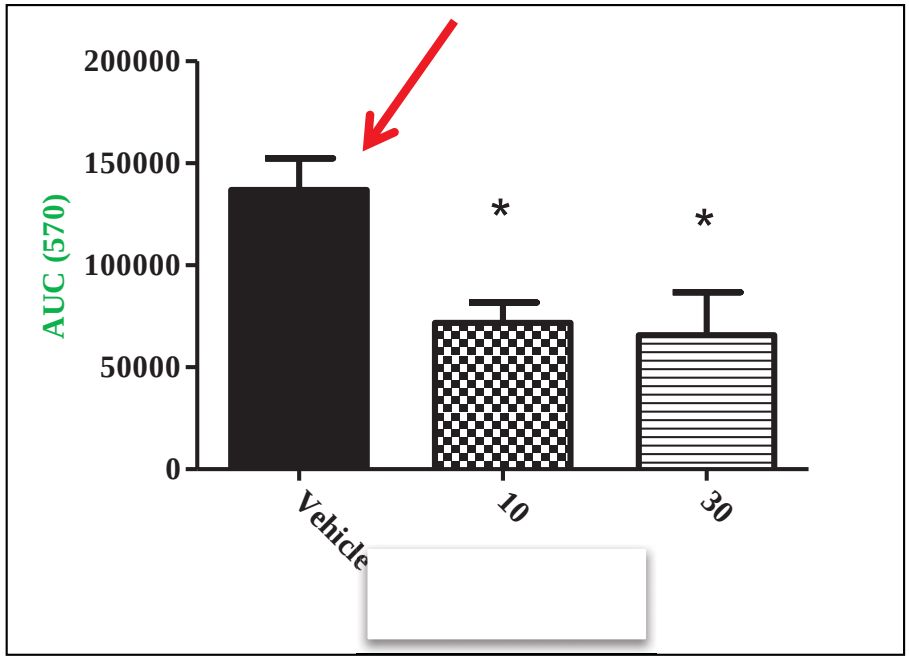
„Outlier“ exclusion

Without “outlier” exclusion



One-way Anova: P= 0.7079

With “outlier” exclusion



One-way Anova: P= 0.0163

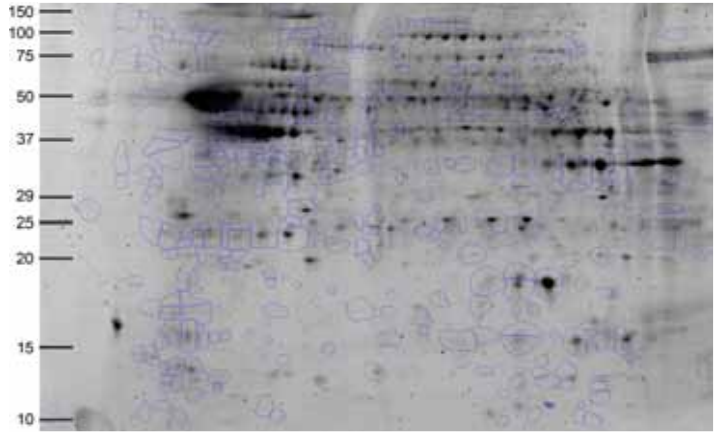
Heads I win, tails you lose

Scenario	Positive control worked	Positive control failed
My drug worked		???
My drug failed		

If one does not pre-specify how the study outcomes will be interpreted and used in decision-making, studies can be designed to bias the interpretation in a favored direction

What if we cannot pre-specify?

24 independent samples (3 x 8)



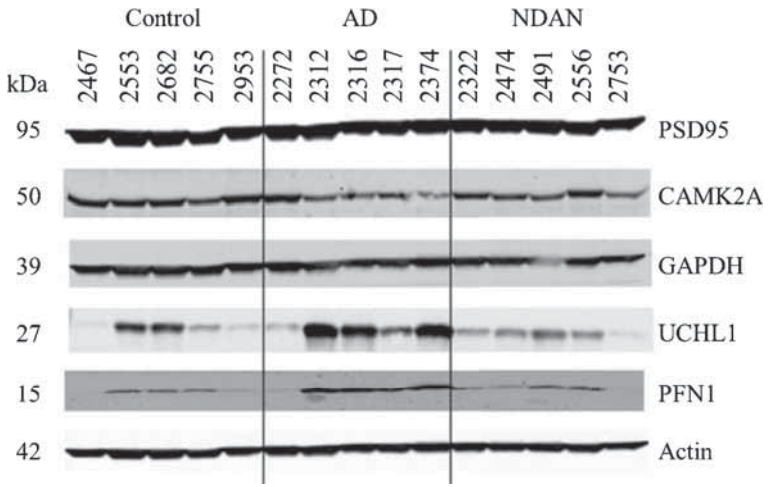
340 most abundant ...

122 with p<0.05 ...

31 with 1.5-fold change or more ...

15 in the final set

15 independent samples (3 x 5)



4 proteins selected for validation

2 proteins confirmed

Conclusion: fifteen proteins which comprise the unique proteomic signature of NDAN PSDs, thus setting them apart from control subjects and AD patients.

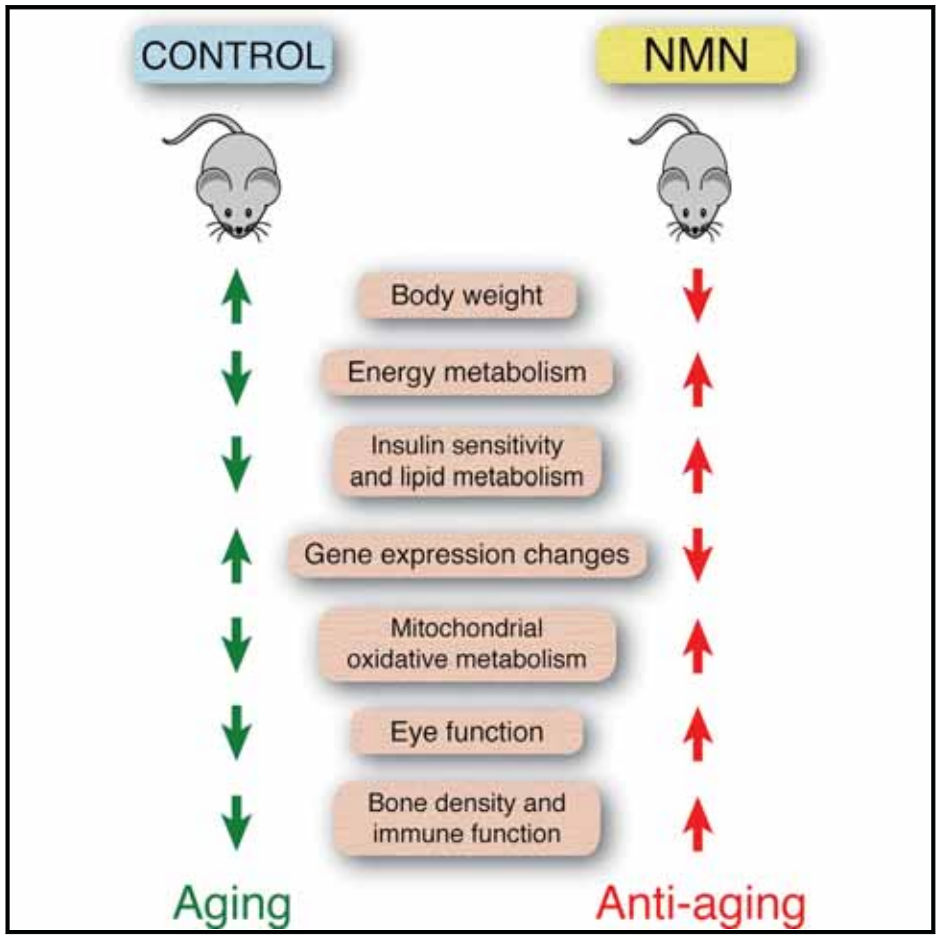
Zolochevska et al (2018) JAD 65: 659–682

Article

Cell Metabolism

Long-Term Administration of Nicotinamide Mononucleotide Mitigates Age-Associated Physiological Decline in Mice

Graphical Abstract



Authors

Kathryn F. Mills, Shohei Yoshida, Liana R. Stein, ..., Koji Uchida, Jun Yoshino, Shin-ichiro Imai

Correspondence

jyoshino@wustl.edu (J.Y.), imaishin@wustl.edu (S.I.)

In Brief

Mills et al. conducted a 12-month-long administration of nicotinamide mononucleotide (NMN), a key natural NAD^+ intermediate, to normal wild-type mice, demonstrating that NMN effectively mitigates age-associated physiological decline in mice without any obvious toxicity. These results highlight the significant potential of NMN as an effective anti-aging intervention in humans.

Can pre-specification be harmful?

Practical issues in preclinical study design

part 3

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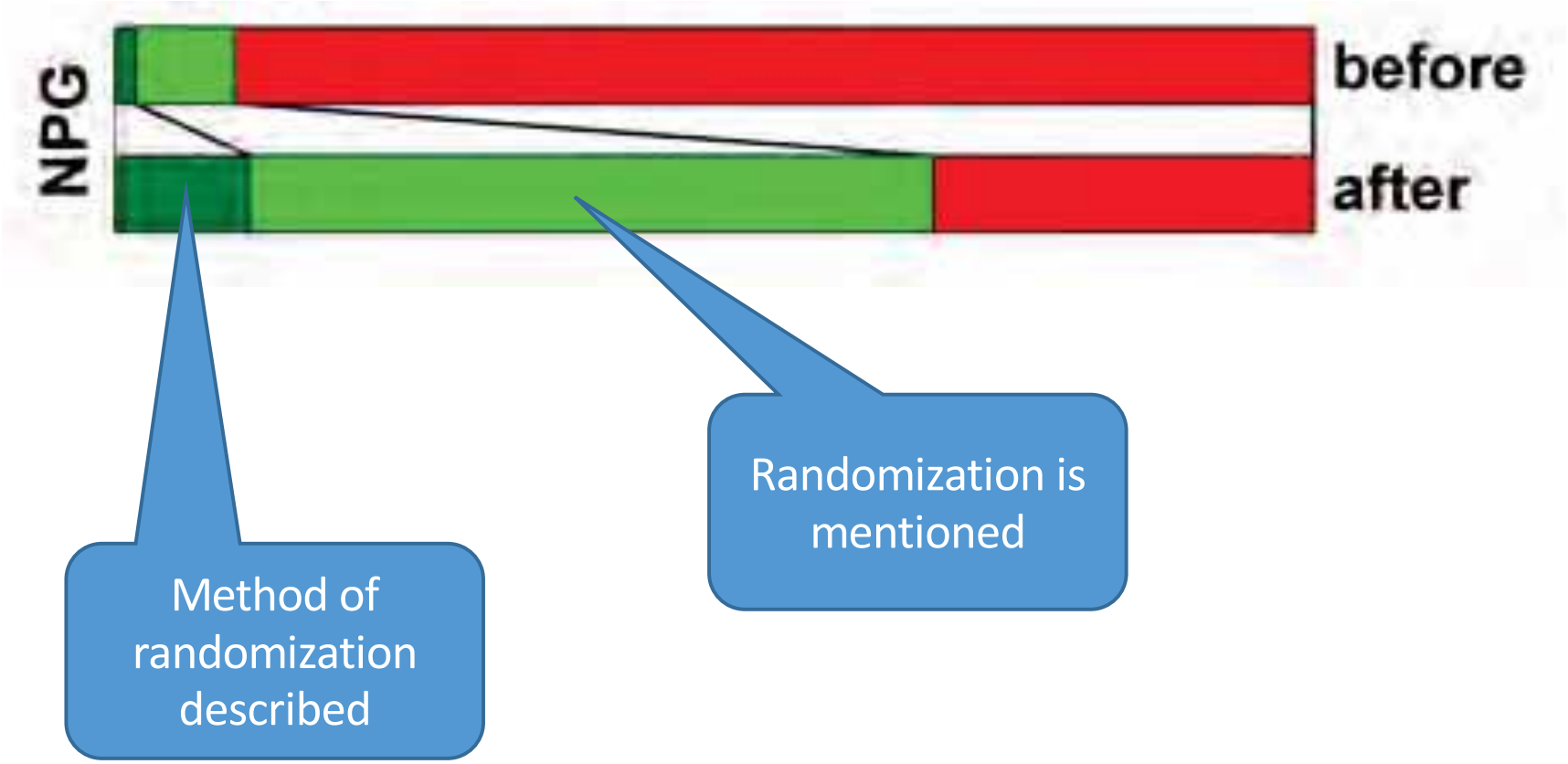
Heidelberg, Germany

PAASP

Randomization

Reporting Checklist For Nature Communications Life Sciences Articles

3. If a method of randomization was used to determine how samples/ animals were allocated to experimental groups and processed, describe it. (Give section/paragraph or page #)



The NPQIP Collaborative group, bioRxiv, 12 Sep 2017

What is randomization?

- Random assignment of subjects to experimental groups / conditions so that:
  - selection bias is prevented
    (i.e. group composition is unpredictable)
  - impact of non-measured confounding factors is minimized
    (i.e. statistically comparable groups are formed)
  - the probability theory can be used to express the likelihood of chance as a source for the difference between groups
  - other bias-reducing measures are enabled
    (i.e. blinding)

„Random“ is the key word!

Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7	Group 8
subj 1	subj 5	…	…	…	…	…	subj 29
subj 2	subj 6	…	…	…	…	…	subj 30
subj 3	subj 7	…	…	…	…	…	subj 31
subj 4	subj 8	…	…	…	…	…	subj 32

Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7	Group 8
subj 1	subj 2	subj 3	subj 4	subj 5	subj 6	subj 7	subj 8
subj 9	subj 10	…	…	…	…	…	…
…	…	…	…	…	…	…	…
…	…	…	…	…	…	subj 31	subj 32

What if it is not random?

- You have to decide whether:
  - Blinding can still be applied
  - Impact of stratification variables can still be evaluated
  - Statistical power is reduced and sample sizes need to be increased
  - Statistical tool can still be applied

- You have to be transparent about it

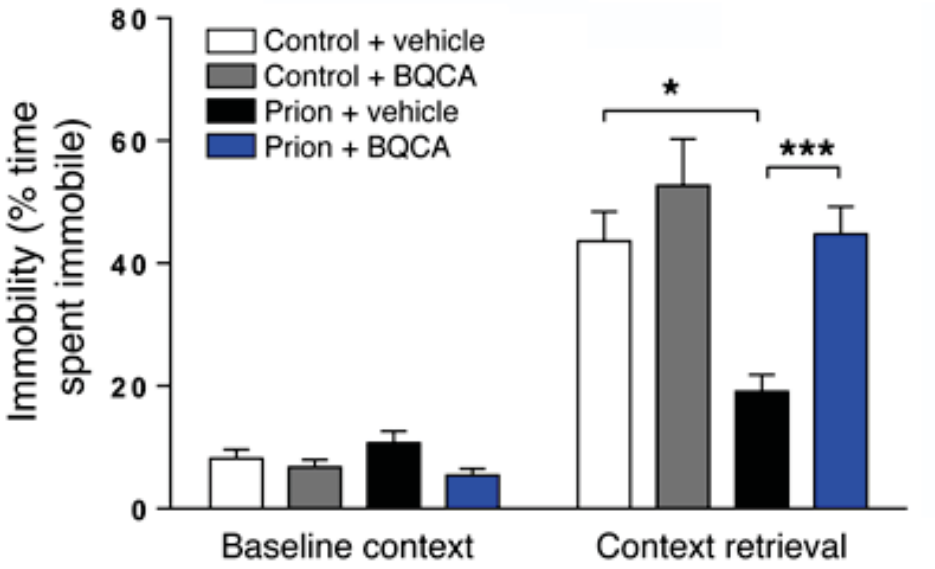
Building a randomization protocol

- Type of randomization
- Tools used for randomization
- Reproducibility of the randomization protocol (i.e. saving the seed of random number generator)
- Methods to monitor / detect deviations from the protocol

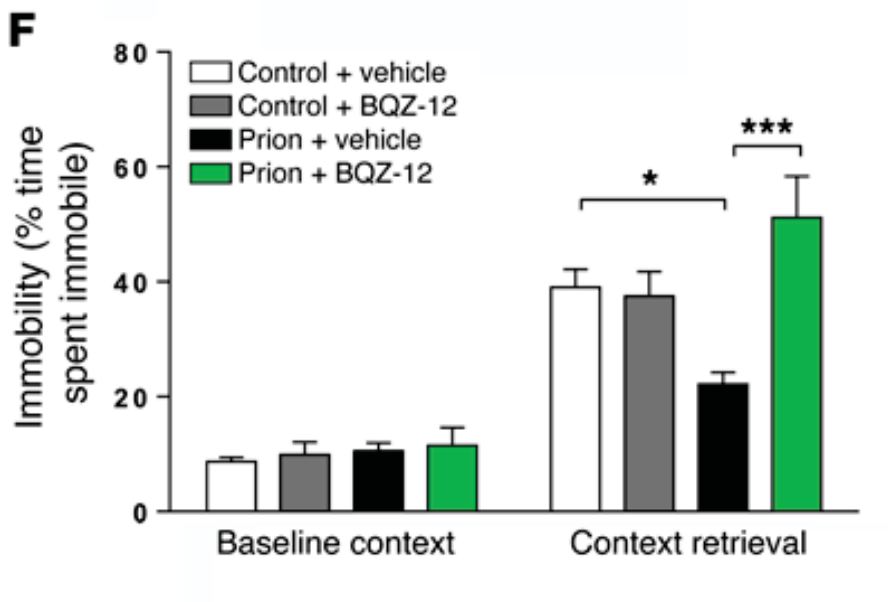
Randomization: Keep is simple?

- For simple experiments with small number of subjects, randomization can be performed by assigning the random numbers from random number tables to the treatment conditions
- Ask for help or use dedicated tools for
  - large sample size situation
  - restricted randomization
  - stratified randomization
  - an unbalanced allocation ratio

Randomization: Keep is simple?



Fear-conditioning response of control and prion-infected mice following administration of vehicle or BQCA (15 mg/kg) 30 minutes prior to training. Mean ± SEM. n = 6–18. *P < 0.05; ***P < 0.001, 1-way ANOVA.



Fear-conditioning response of control and prion-infected mice following administration of vehicle or BQZ-12 (1.5 mg/kg) 30 minutes prior to training. Data are shown as mean ± SEM. n = 12–19

Bradley et al (2016) JCI

Simple randomization

- Equivalent to tossing a coin for each subject that enters a study
  - in preclinical studies, usually all subjects are known at the start of a study
- Can be done with the random number generator
  - http://www.randomizer.org/
  - MS Excel
- Treatment assignment is unpredictable
  - but can get imbalanced in treatment assignment, especially in smaller trials

Simple randomization

- MS Excel function - RANDBETWEEN(0.5;4.5)

Subject ID	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Group ID	4	1	1	3	3	1	4	4	3	4	3	3	4	2	3	1

- MS Excel function - RAND ()

Treatment	1	1	1	1	2	2	2	2	3	3	3	3	4	4	4	4
Random number	.76	.59	.51	.90	.64	.10	.50	.48	.22	.37	.05	.09	.73	.83	.50	.43

Subject ID	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Treatment	3	3	2	3	3	4	2	2	4	1	1	2	4	1	4	1
Random number	.05	.09	.10	.22	.37	.43	.48	.50	.50	.51	.59	.64	.73	.76	.83	.90

Block randomization

- Used to fix the balancing problem of simple randomization
- Tools available or can be easily developed

- Step 1: define the block size
  - Minimum block size: number of treatment conditions
  - Block size can be equal to number of animals per cage

- Step 2: identify possible treatment allocations within a block
  - Two treatments (A,B)
  - If block size equals 2, possible treatment allocations
    (1) AB, (2) BA
  - If block size equals 4, possible treatment allocations
    (1) AABB, (2) BBAA, (3) ABAB, (4) BABA, (5) ABBA, (6) BAAB

Block randomization

- Step 3: randomize blocks with varying treatment allocations

Block number	4	3	1	6	5	2
Random number	.015	.379	.392	.444	.720	.901

- Step 4: Assign subjects to groups

Block number	4				3				1				6			
Random number	.015				.379				.392				.444			
Subject ID	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	-
Treatment	B	A	B	A	A	B	A	B	A	A	B	B	B	A	A	-

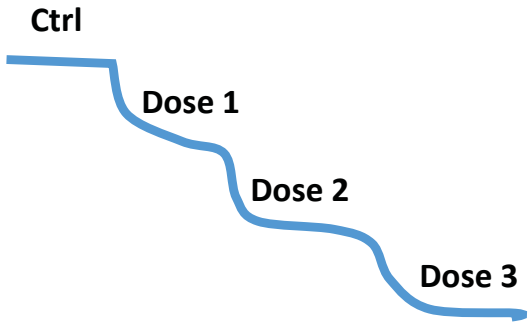
- Step 5 (optional): Blind the block size

Randomization: Challenges

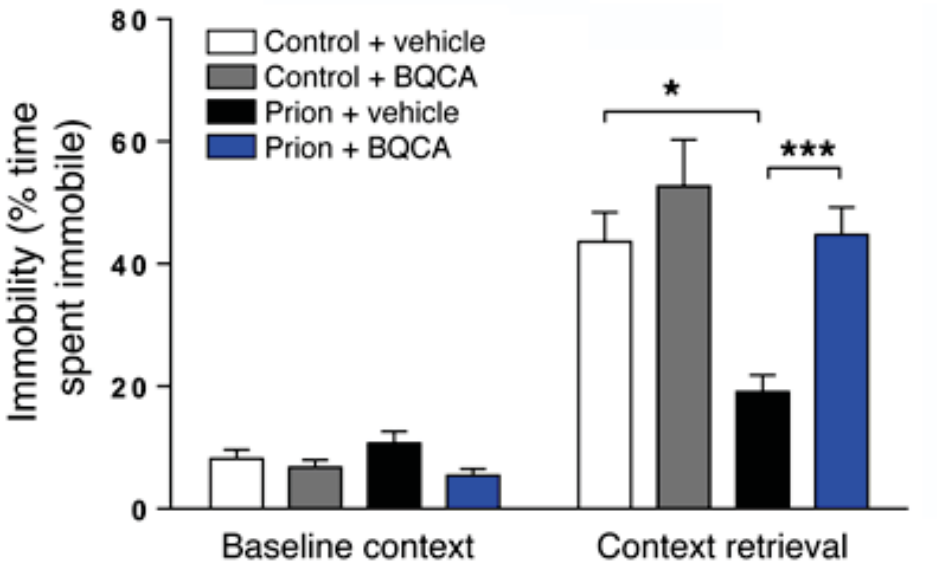
- No “one-size-fits-all” solution
- Small sample sizes
- Group-housed animals
- Cross-contamination (drug treatment)
- Cross-over designs
- Pseudo-randomization
- Perceived complexity of implementation
- Randomization not maintained until the end
- Exceptions …

Randomization: Special cases

- Cumulative dosing, e.g.
  - Cardiovascular safety testing
  - Single unit recordings in electrophysiology
- Randomization after the start of the experiment, e.g.
  - Treatment groups are formed after tumor growth is evidence and randomization is based on tumor size or other clinical signs



Can randomization be harmful?



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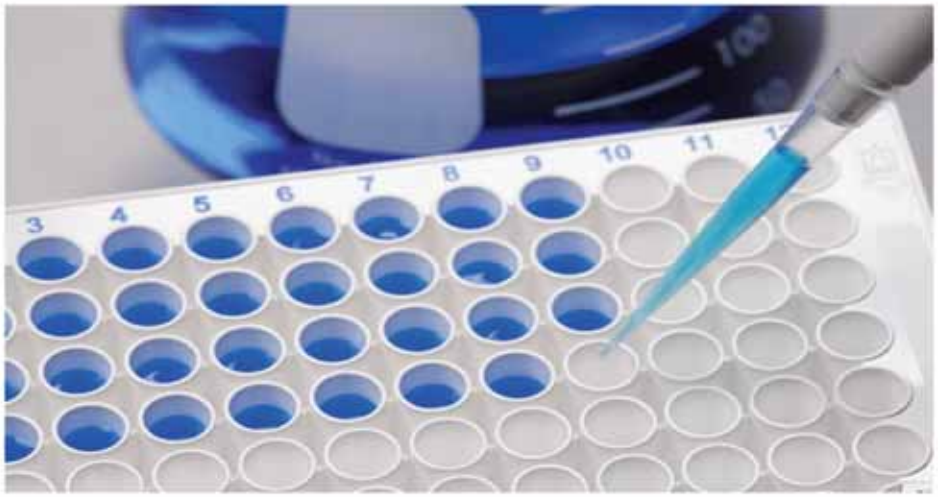


Practical issues in preclinical study design

part 4

Anton Bespalov

Partnership for Assessment and Accreditation of Scientific Practice

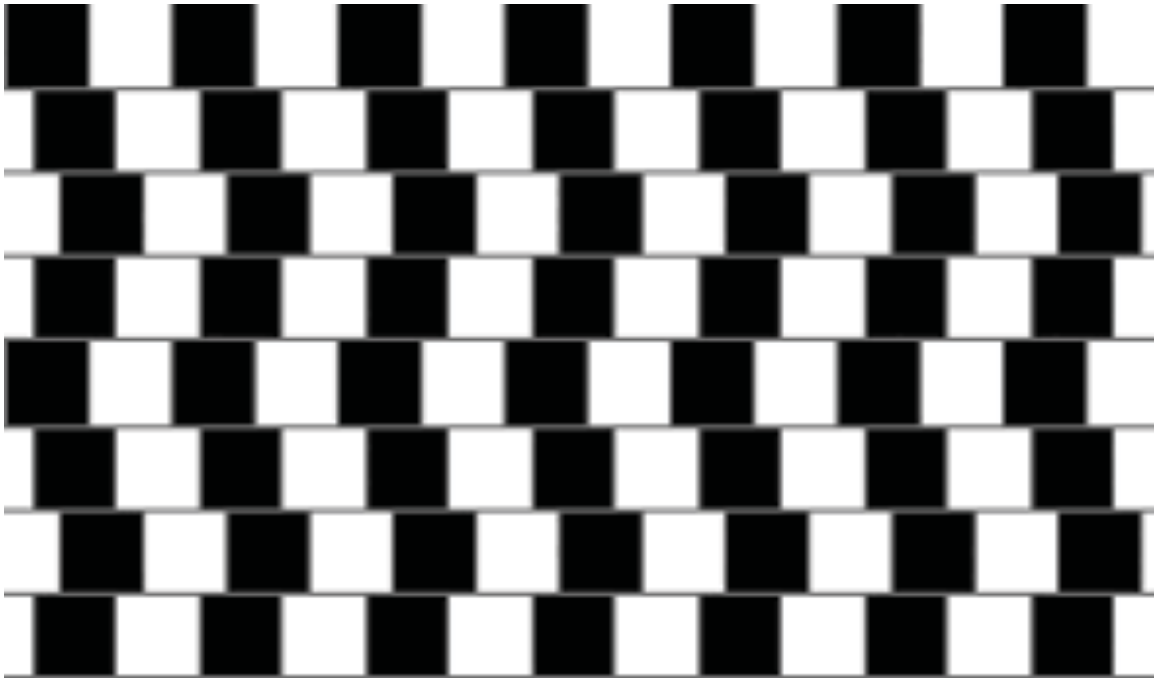
Heidelberg, Germany

PAASP

Blinding

What is blinding?

- Concealment of treatment group allocation from one or more individuals involved in a research study
- Aims to reduce or eliminate bias, until after the study results are known
- Bias may be intentional or unconscious, thus no dishonesty is implied by blinding



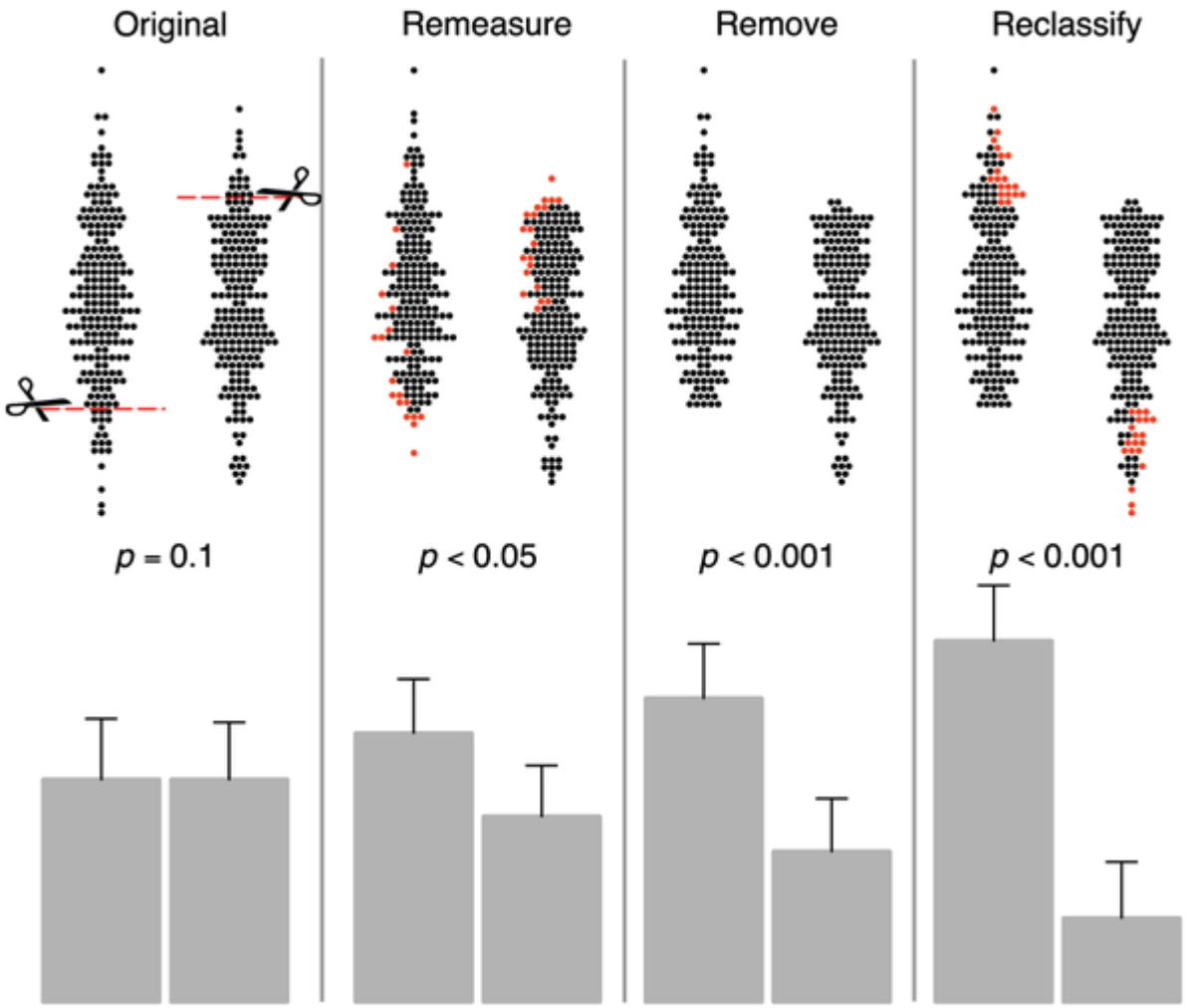
Breaking the blind

- In clinical studies, patients and investigators often play a game in double-blind clinical trials
  - look for clues to help them identifying the treatment a patient is receiving
  - in multi-site trials with few patients per site, reward is primarily inner satisfaction
- Although it may look innocent, generates biases that can affect the outcome!

Breaking the blind

- In preclinical studies, breaking the blind certainly affects the outcome
  - Preclinical studies are rarely supported by clearly pre-specified criteria and guidance on how to treat various accidents during the experiment or deviations from the original protocol
  - Hence, ”abnormal” values recorded for one of the study subjects may be attributed:
    - to treatment (and then would be kept), or
    - to those accidents (and would be discarded)

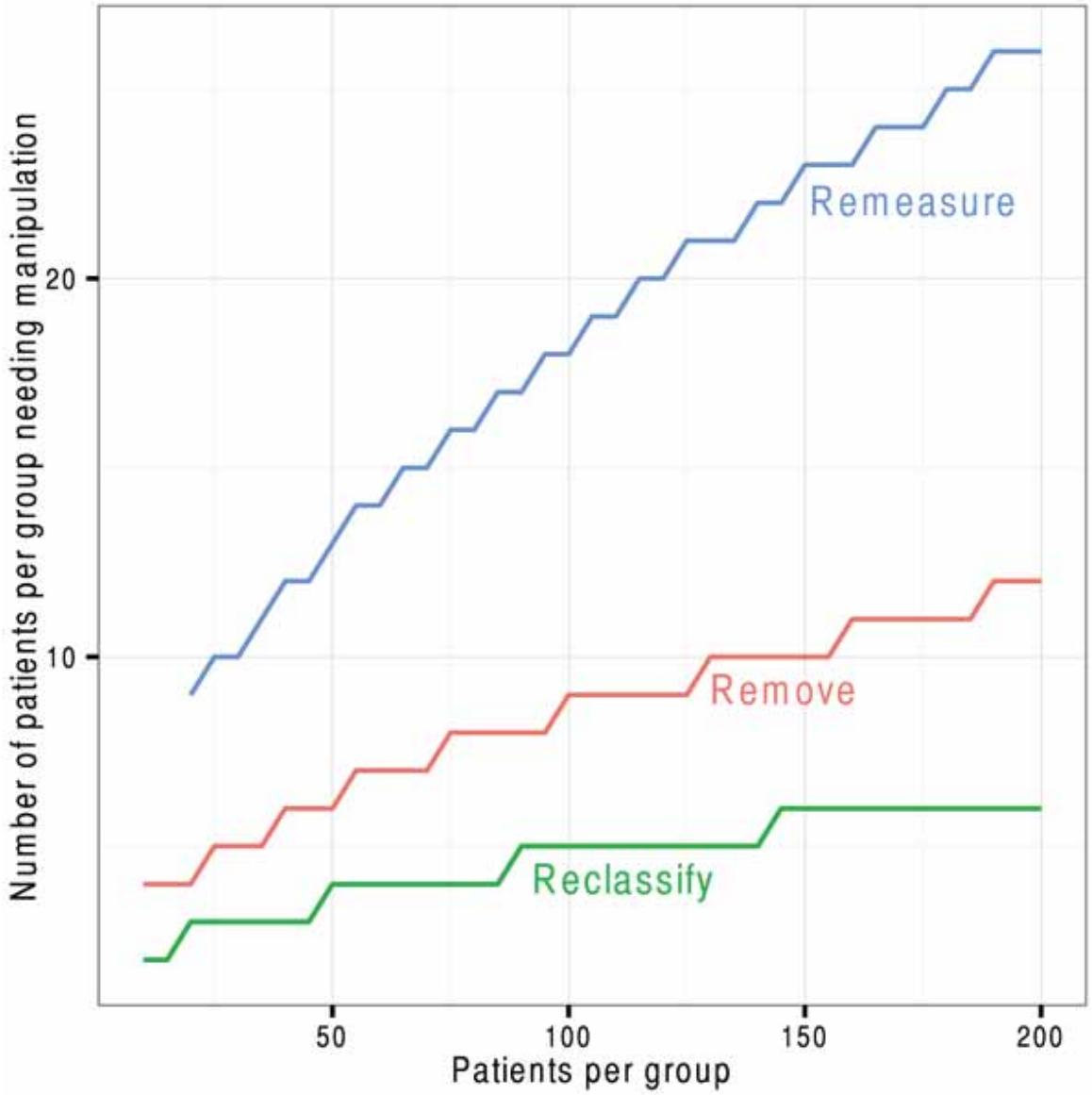
Three evil Rs



A researcher, who believes that two groups differ may, at the time of acquiring a surprising value in an individual subject may elect to remeasure, remove, or reclassify the subject

Shun-Shin & Francis (2013)
PLOS ONE 8(6): e65323

Three evil Rs have an impact!

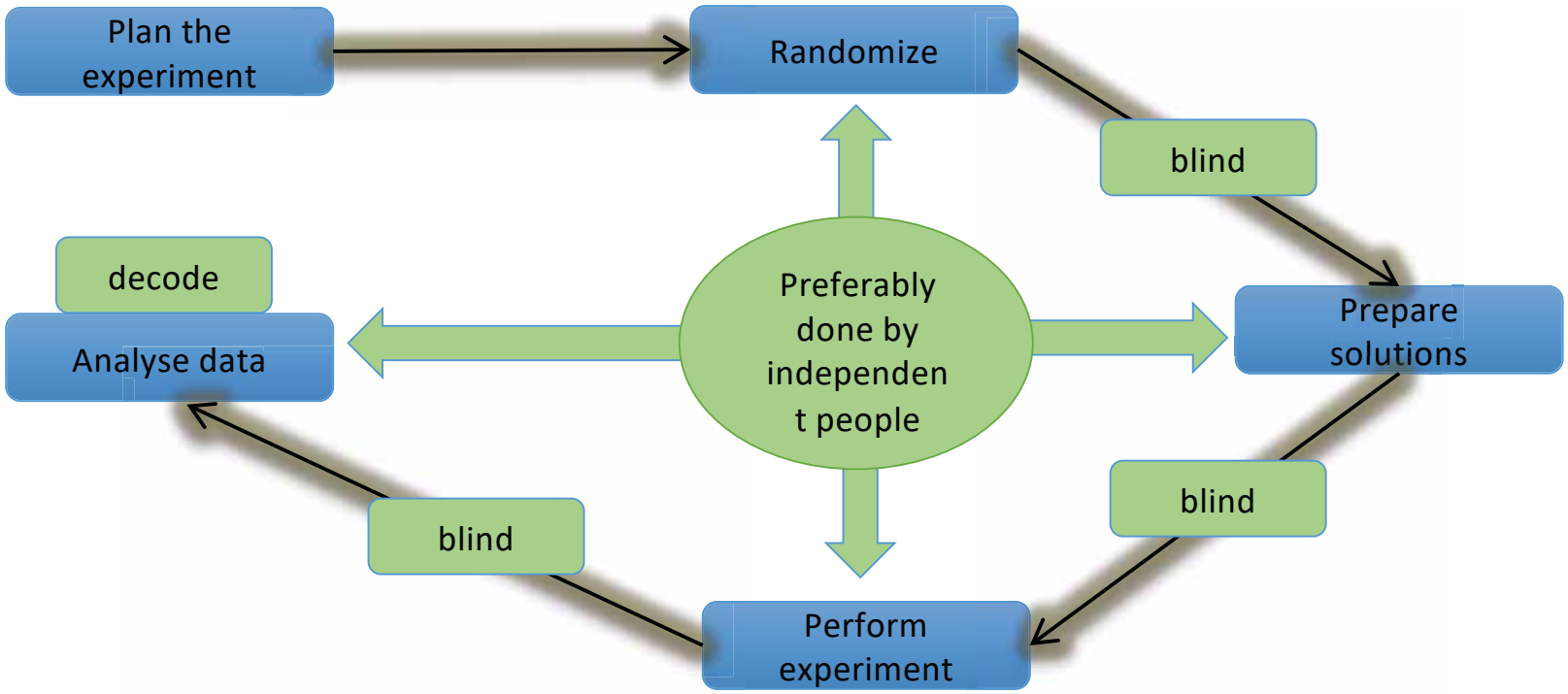


The number of patients per group needing to be remeasured, removed, or reclassified to make an otherwise neutral study positive by the Mann-Whitney U-test

Shun-Shin & Francis (2013)
PLOS ONE 8(6): e65323

Blinding in preclinical studies

- An integral part of the good research practice efforts and will not be effective if implemented as a standalone measure
- Instead of single- and double-blind studies, full and partially blinded studies
- Can have a major impact on throughput and, therefore, is often not even considered


Plan the experiment
Randomize
blind
Prepare solutions
blind
Perform experiment
blind
Analyse data
decode
Preferably done by independen t people

Cost of blinding: How to handle that?

- Fit-for-purpose blinding

Levels of blinding

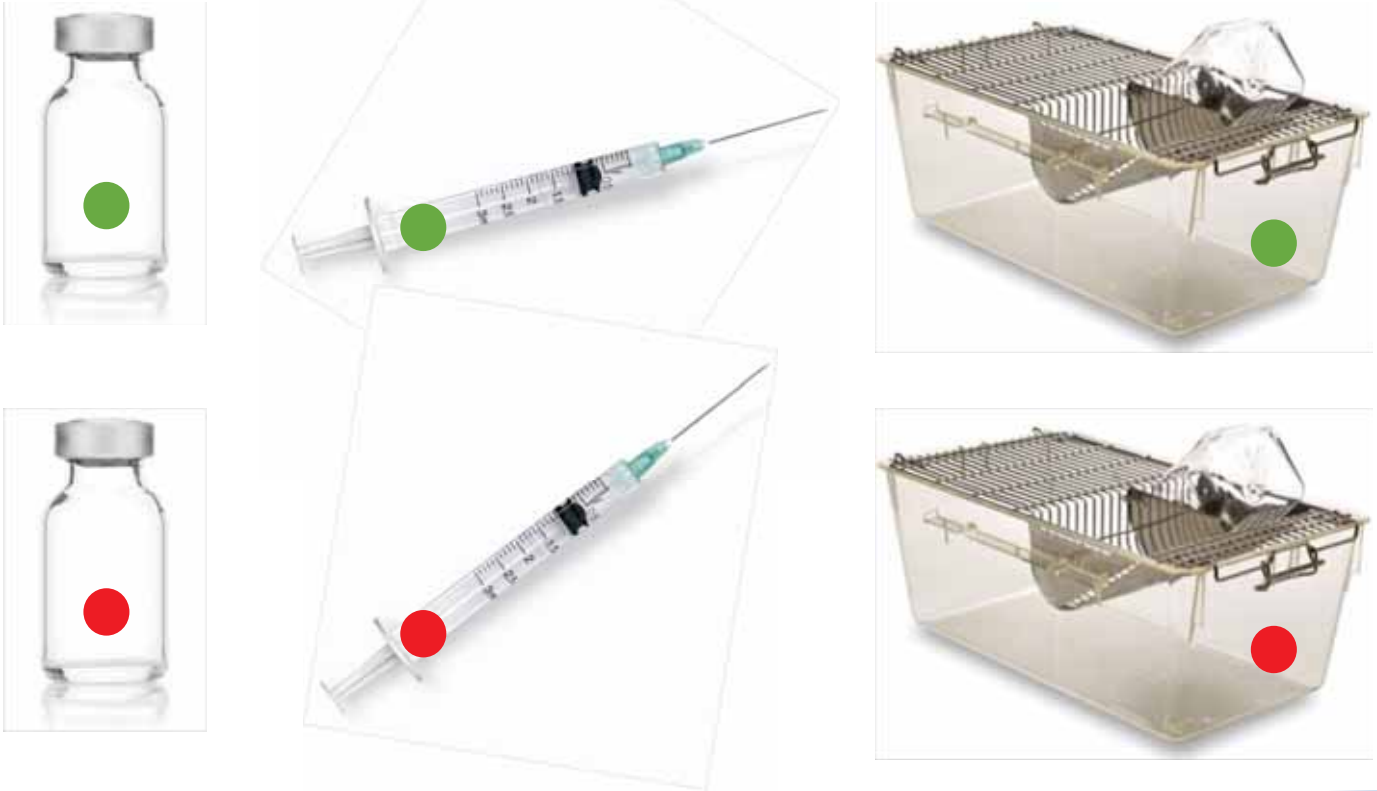
- Level 1 – „assumed“ blinding or no blinding
  - Suited for exploratory research and for routine experiments that cannot be later converted into ”decision enabling” unless repeated under higher level of blinding
  - Planning, injection, performance, and analysis often by one person

- Level 2 – partial blinding (blind analysis)
  - Applicable when critical data collection or analysis is done separately from the general (preparatory) experimental phase (e.g. image analysis in neuroanatomy after the in-life phase is finished or behavioral analysis of videorecordings)
  - Analysis is done by independent person(s)

- Level 3 – full blinding
  - Required for PoC and decision-enabling studies
  - Independent people responsible for:
    - (de)coding, randomization
    - injections, (performance)
    - (performance), analysis

Cost of blinding: How to handle that?

- Fit-for-purpose blinding
- Techniques and methods that make it easier to do



Cost of blinding: How to handle that?

- Fit-for-purpose blinding
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- Engage lab associates, students and bench scientists

Cost of blinding: How to handle that?

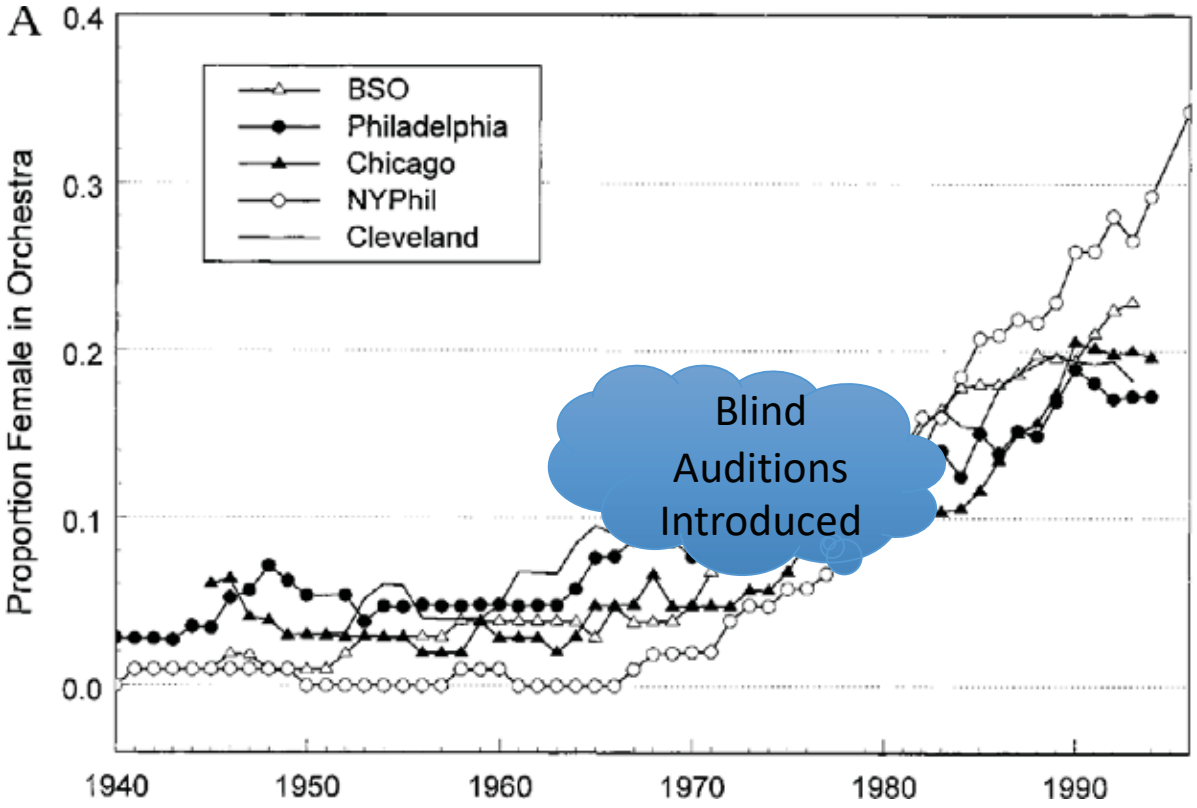
- Fit-for-purpose blinding
- Techniques and methods that make it easier to do
- Engage lab associates, students and bench scientists
- Seek support from outside (from neighbouring departments to dedicated funding)

Blinding: Why do we resist?

- “Trust” issue
- Resource requirements to implement proper blinding
- Awareness of the “negative” consequences of properly blinded studies

Zubin Mehta is credited with saying,
"I just don't think women should be in an orchestra."

www.curt-rice.com



Goldin & Rouse (2000) American Economic Review 90: 715

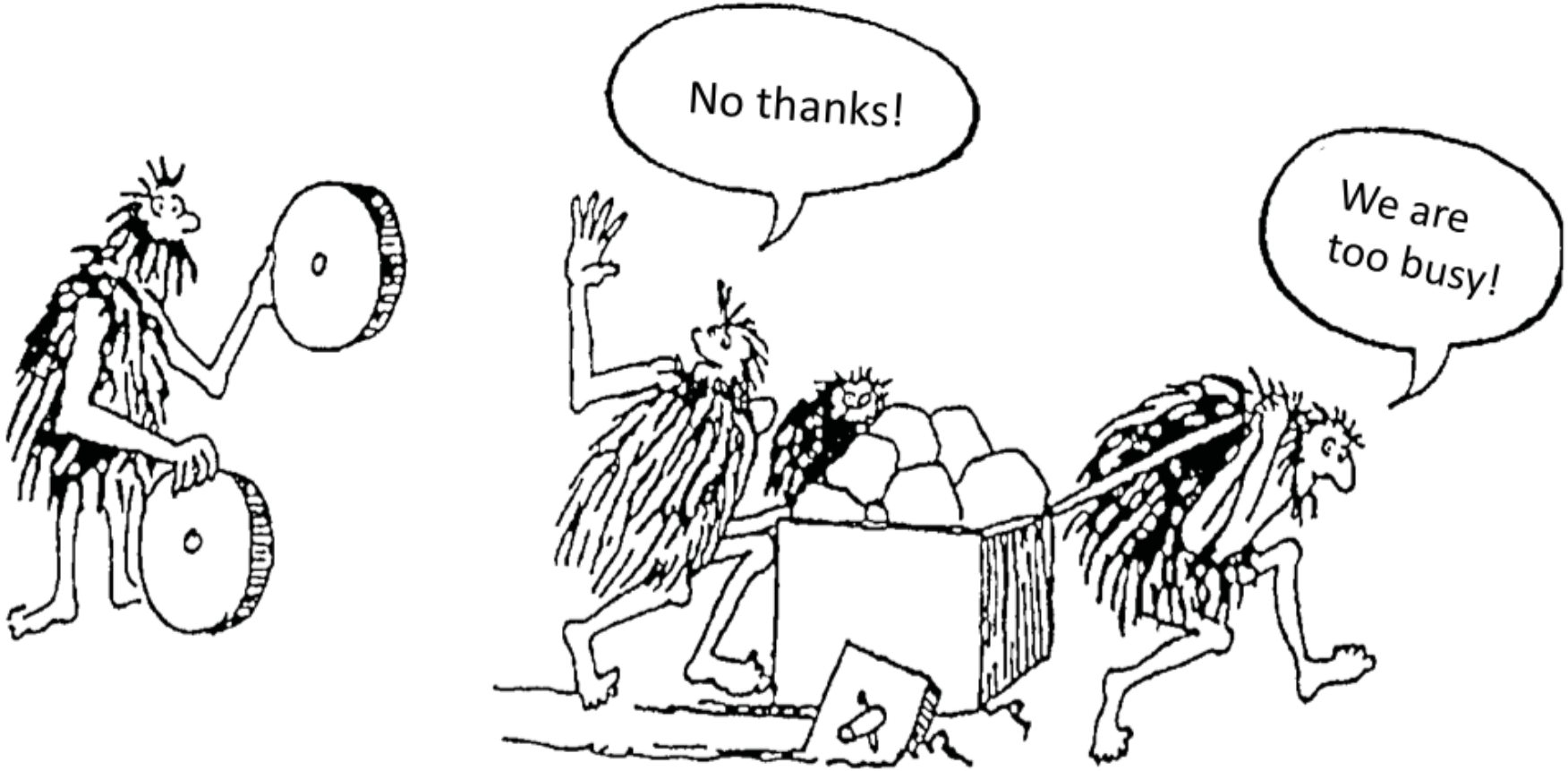
Blind auditions (jury and candidates are separated by a curtain) explain ca. 30% of the increase in the female proportion of "new hires" at major symphony orchestras in the US

Can blinding be harmful?

- Animal welfare and emergency decoding
- Risk of mistakes

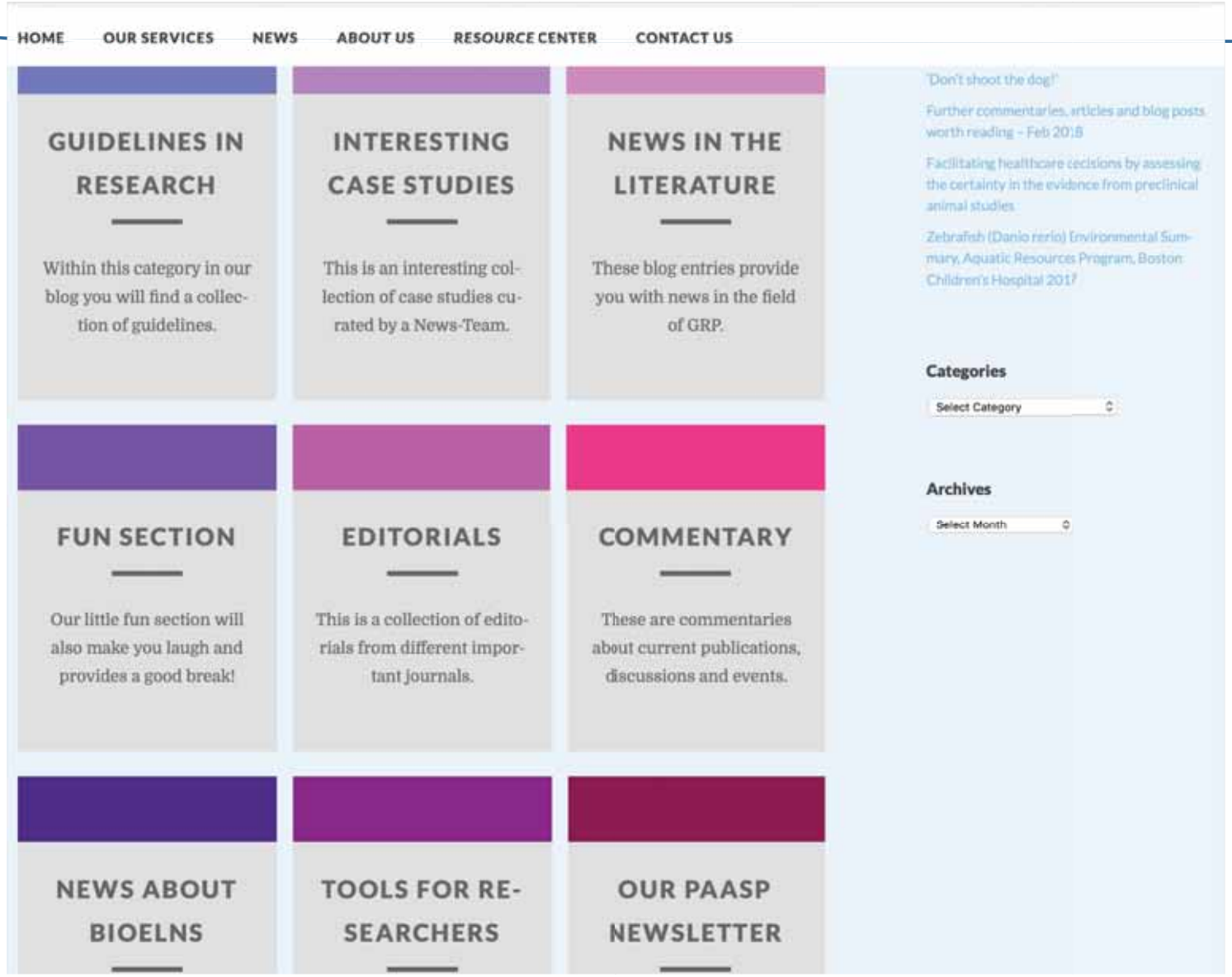
Being transparent about randomization and blinding: Minimum requirements

Procedure	Technical report / Laboratory notebook record	Scientific publication
Randomization	Type of randomization Block size (if applicable) Stratification variables (if applicable) Tools used for randomization Reference to the protocol followed Deviations from the protocol (if any)	Type of randomization Tools used for randomization Stratification variables (if applicable)
Blinding	Type of blinding Records of unblinding (if applicable) Reference to the protocol followed Deviations from the protocol (if any) Colleague(s) who provided blinding	Type of blinding Statement whether blinding integrity was maintained Statement whether blinding was provided by one of the co-authors



www.vide.se

Resource center @ paasp.net



THANK YOU!