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CNR Area della Ricerca
di Pisa

Corso di formazione in materia di protezione degli animali utilizzati a fini scientifici

Accreditato dal Ministero della Salute secondo D.M. 5 agosto 2021 e D.D. 18 Marzo 2022

15 giugno 2023

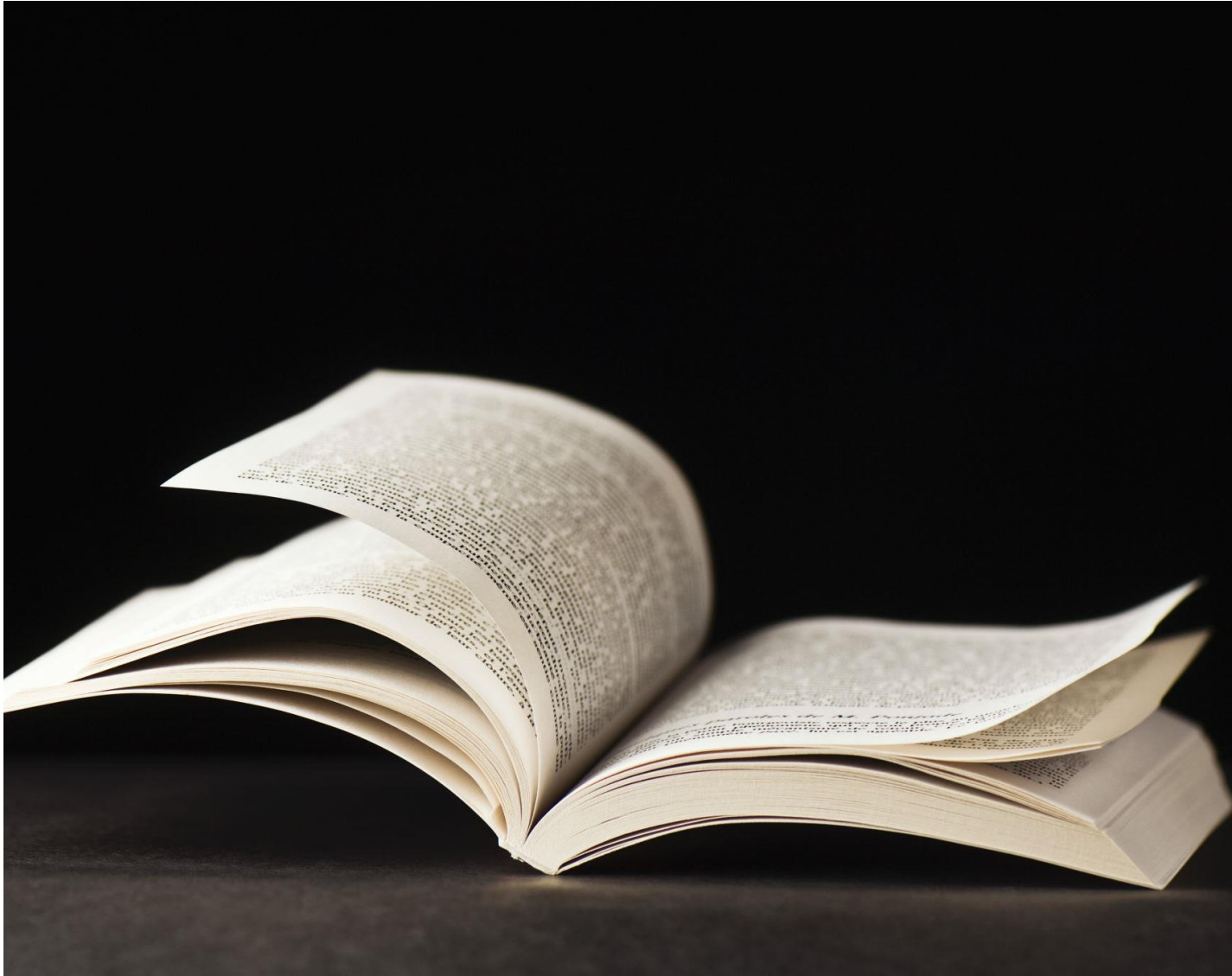
Modulo 2

Etica, benessere degli animali e Tre R (livello 2)



Centro 3R

Docente: Arti Ahluwalia. Università' di Pisa e Centro 3R



“Alternatives”?

- The word was used by Burch, but Russell considered it sounded like *Replacement*
- It is not used in their book.

- The term was used in a paper by J Hegarty (Treasurer of FRAME) in 1971 and (for all the 3Rs) by DH Smyth in his book *Alternatives to animal experiments* (1978).

- Some now talk about
 - *Replacement alternatives/NATs/NAMs*
 - *Reduction alternatives*
 - *Refinement alternatives*

- *But largely and especially for the public, **Alternatives** are synonymous with non animal substitutes*

Russell and Burch's original definition of the 3RS:



- **Replacement:** *any scientific method employing non-sentient material which may in the history of animal experimentation replace methods which use conscious living vertebrates*
- **Reduction:** *means of minimising, other than by Replacement, the number of animals used to obtain information of a given amount and precision*
- **Refinement:** *measures leading to a decrease in the incidence or severity of inhumane procedures applied to those animals which have to be used.*

Now more emphasis on **welfare benefit** and **knowledge gain** as well as minimising inhumanity

Also **validity**

	Basic	Updated
Replacement	Avoiding or replacing the use of animals in areas where they otherwise would have been used.	Accelerating the development and use of predictive and robust models and tools, based on the latest science and technologies, to address important scientific questions without the use of animals.
Reduction	Minimising the number of animals used consistent with scientific aims.	Appropriately designed and analysed animal experiments that are robust and reproducible, and truly add to the knowledge base.
Refinement	Minimising the pain, suffering, distress or lasting harm that research animals might experience.	Advancing research animal welfare by exploiting the latest <i>in vivo</i> technologies and by improving understanding of the impact of welfare on scientific outcomes.

Reduction

- **Reduction:** methods for obtaining comparable levels of information from the use of fewer animals in scientific procedures, or for **obtaining more information from the same number of animals**
- Perhaps obtaining even **better** information....
- It's all about **Optimisation** of animal numbers:
 - fewer animals (if possible)
 - more information from the same number of animals
 - *more* animals (if the original suggestion was too low to achieve conclusive results)
- *Too few animals can lead to false conclusions and is a waste of animal lives and human resources.*

Refinement

Methods that

- minimise pain, suffering, distress and lasting harm
- maximise animal welfare

All the way from procurement of the animals to humane killing or other outcomes (e.g. re-use, rehoming)



An enormous scope for refinement

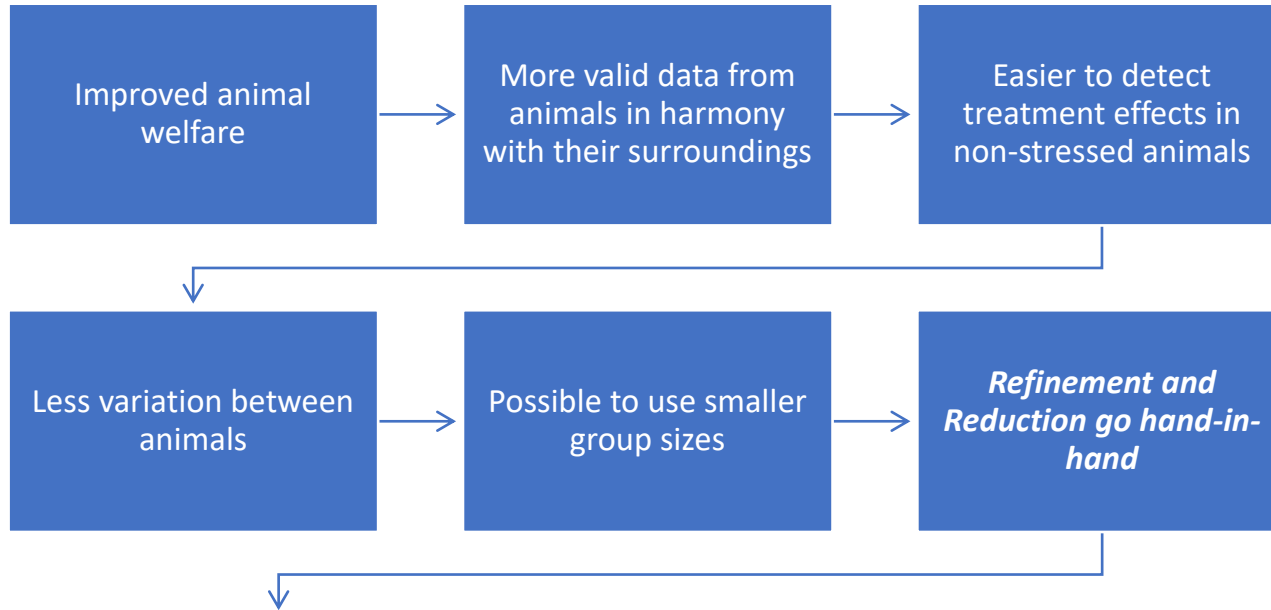
e.g. housing, environmental enrichment, handling, dosing, sampling, anaesthesia and analgesia, less sentient animals

Refinement

- What is the difference between reduction and refinement?

Reduction and Refinement are actually inseparable

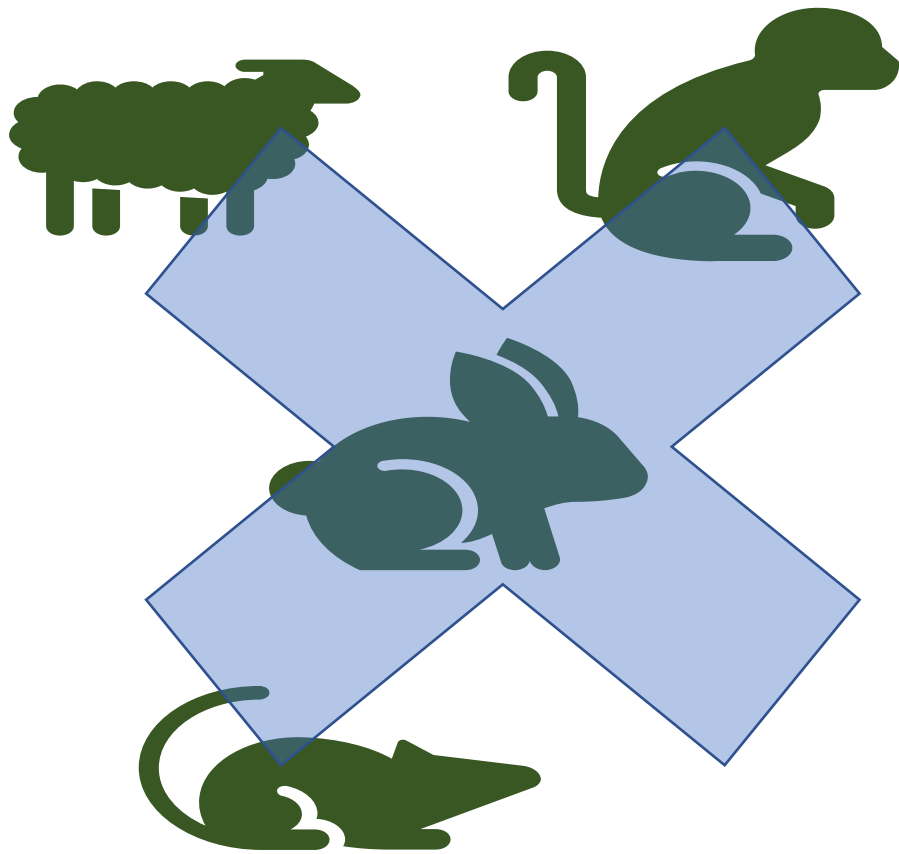
Refinement: win-win



colourbox.com

‘Happy animals make good science’
(Poole, 1997)

EU 2010/63:



Research on non vertebrates

IT 2014/26 Article 5, points 2e: *“procedures cannot be authorized for research on substances of abuse”*

When studying the neurobiology of addiction in planarians, c. elegans, honey bees, crayfish and drosophila, investigations are conducted on adaptive behaviour, locomotion, molecular or genetic typing

The same types of studies are also conducted on mice

Notable parallels with mammalian systems at the behavioural, neuro-chemical, and molecular levels

Neurobiology: behavioural studies

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Proceedings of the National Academy of Sciences

Neuroscience and Biobehavioral Reviews 120 (2021) 1–12



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Neuroscience

Ancestral regulatory mechanisms specify conserved and vertebrates

[Jessika C. Bridi](#)^{a,b,1,2} [Zoe N. Ludlow](#)^{a,b,1} [Benjamin Kottler](#)^{a,b} [Beat](#)
[Jonah Dearlove](#)^{a,b} [Markus Göker](#)^e [Nicholas J. Strausfeld](#)^{f,g,3} [Patr](#)

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Review article

Rodent and fly models in behavioral neuroscience: An evaluation of methodological advances, comparative research, and future perspectives

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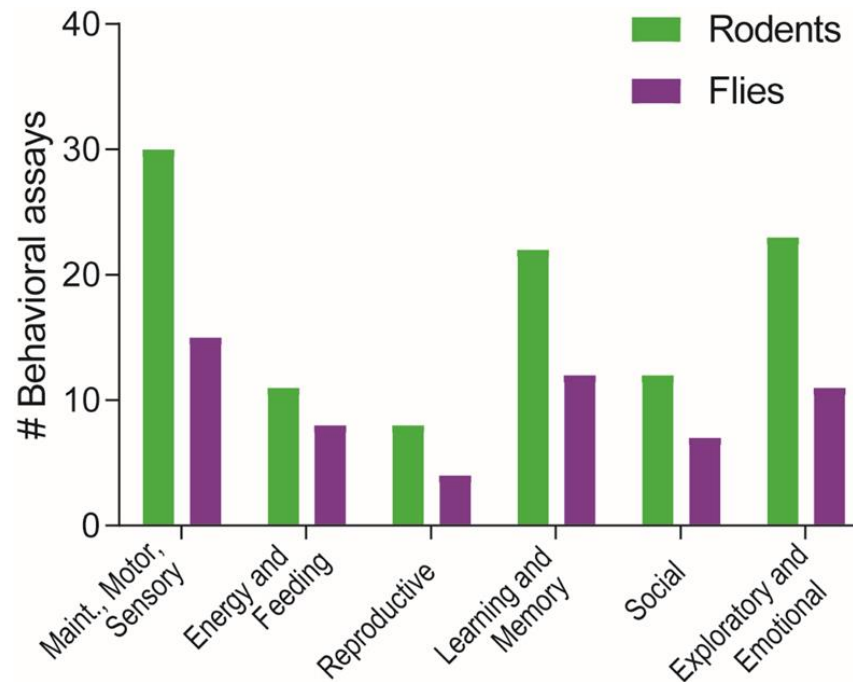
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“conserved regulatory mechanisms specify brain circuits for sensory integration and coordinated behavior common to all animals that possess a brain”

The same type of study

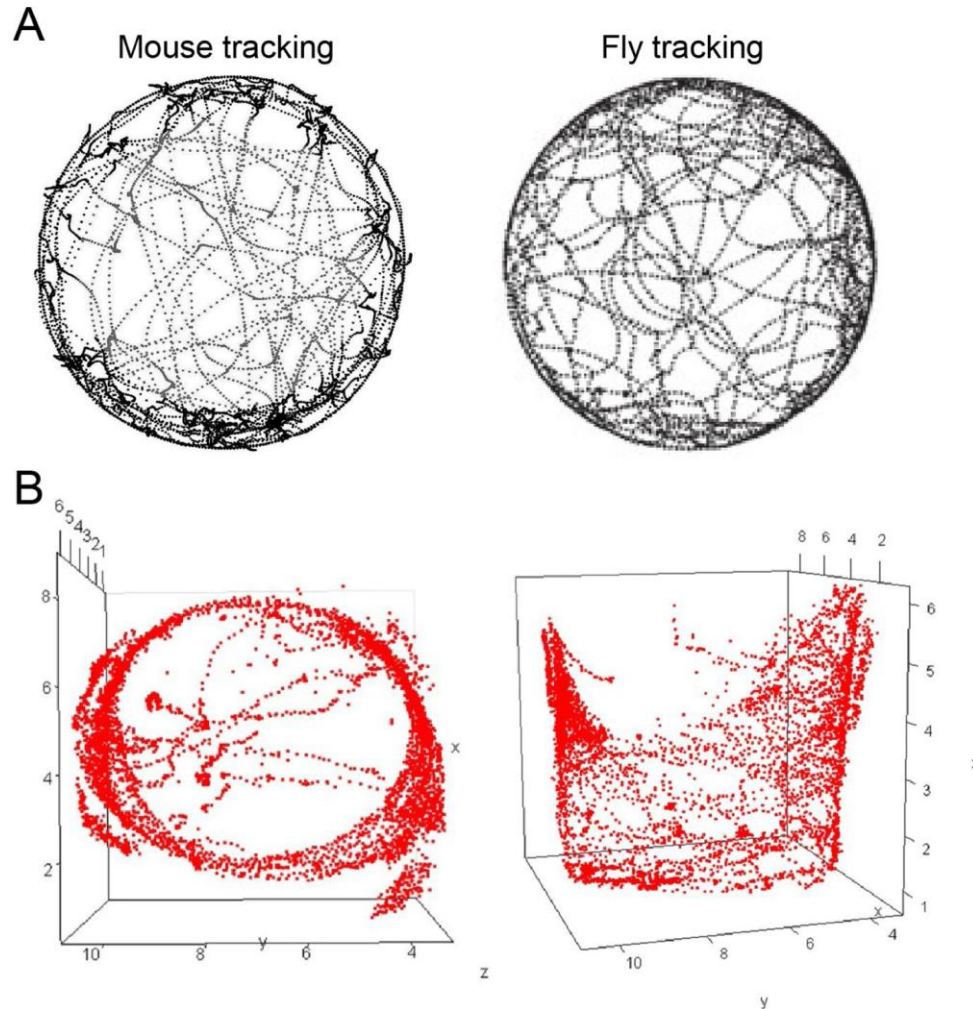
Basic neurobiology assays



Analysis of popular behavioral assays for mice and flies. Based on a comprehensive literature search, currently performed assays for each model were identified, cataloged, and grouped in accordance to six main sub-fields of behavioral assessment. In all fields, a higher number of tasks were developed for rodents; however, the proportion of assays between categories is the same for both models (chi-squared test, $p = 0.986$).

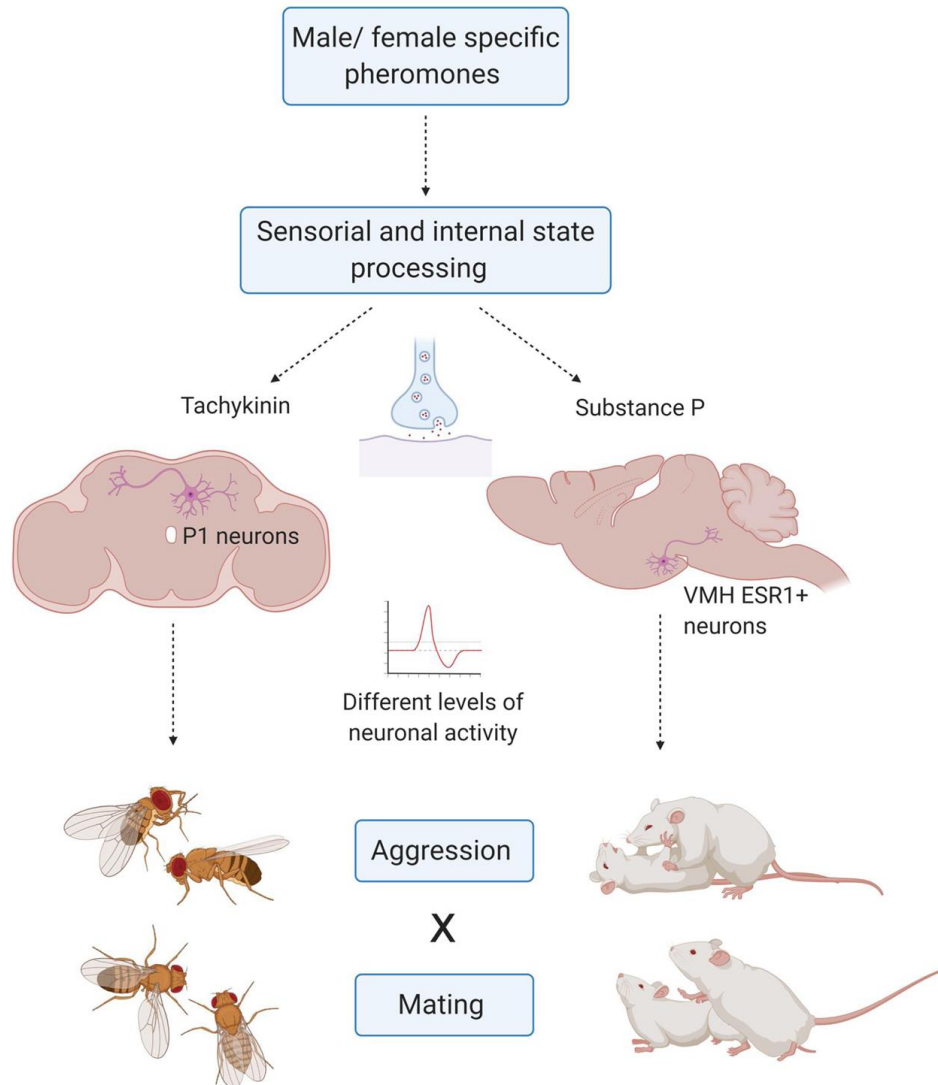
Very similar results

Locomotion assays



Similarities in open field behavior between rodents and flies. (A) Representative locomotion tracking records of mouse (left panel, adapted from Zhan, 2015) and *Drosophila melanogaster* (right panel, adapted from White et al., 2010). (B) Tridimensional tracking records of the locomotion of a fly (retrieved from Ardekani et al., 2012).

Similarity in regulation and circuitry organization: neuronal populations receive inputs from homologous neuropeptide pathways and promote internal states that modulate behaviours



Circuitry similarities for aggression and mating behaviors across models. Both flies and rodents process sex-specific pheromones and environmental clues, which culminates in the stimulation of specific neuronal populations that govern the choice between aggression and mating. The activity level of these cells, which express evolutionarily-conserved receptors, mediates the behavioral output. (Moulin et al, 2021)

Reduction

- How to reduce?
- Experimental design, statistical methods



Reproducibility crisis!



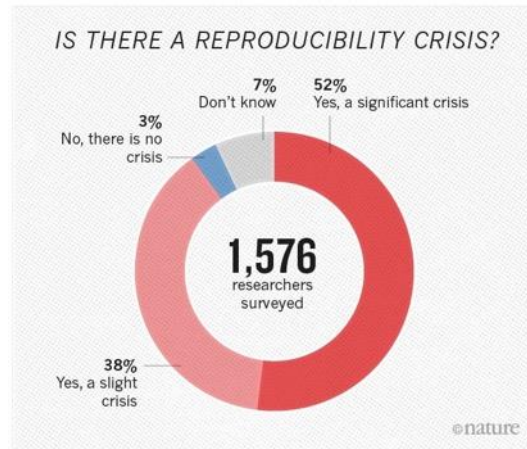
NATURE | NEWS FEATURE

1,500 scientists lift the lid on reproducibility

Survey sheds light on the 'crisis' rocking research.

Monya Baker

25 May 2016 | Corrected: 28 July 2016



More than 70% of researchers have tried and failed to reproduce another scientist's experiments, and more than half have failed to reproduce their own experiments. Those are some of the telling figures that emerged from *Nature's* survey of 1,576 researchers who took a brief online questionnaire on reproducibility in research.

NATURE | NEWS

Swiss survey highlights potential flaws in animal studies

Poor experimental design and statistical analysis could contribute to widespread problems in producing preclinical animal experiments.

Ramin Skibba

20 December 2016

Pain management in pigs undergoing experimental surgery; a literature review (2012–4) FREE

A. G. Bradbury, M. Eddleston, R. E. Clutton ✉

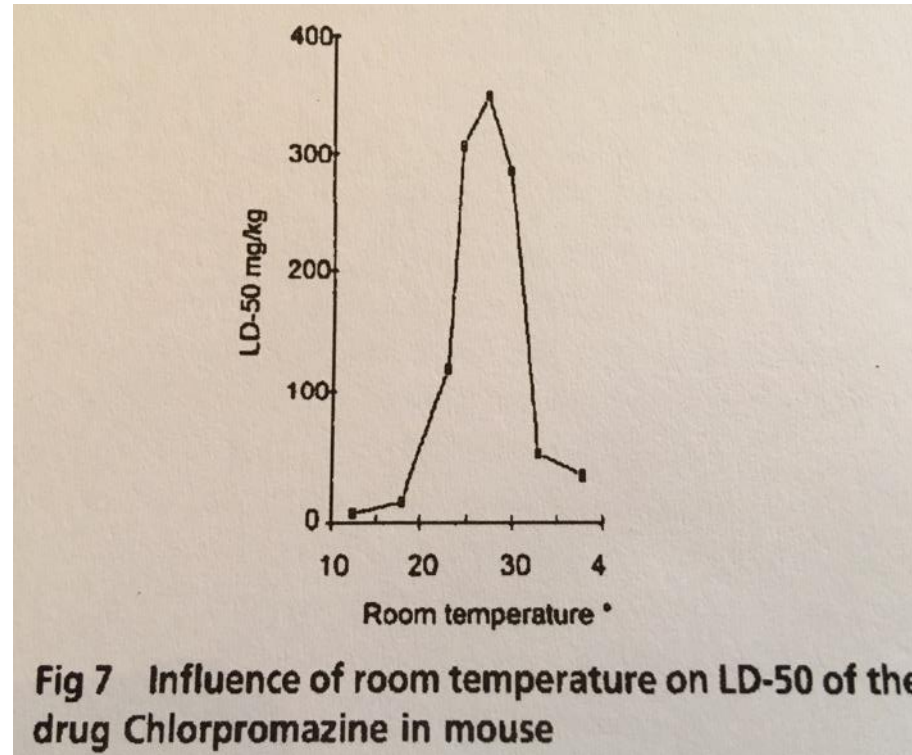
Br J Anaesth (2016) 116 (1): 37-45. DOI: <https://doi.org/10.1093/bja/aev301>

Published: 03 October 2015

with analgesic properties, but only 87/233 (37%) described postoperative analgesia. No article provided justification for the analgesic chosen, despite the lack of guidelines for analgesia in porcine surgical models and the lack of formal studies on this subject. Postoperative pain assessment was reported in only 23/233 (10%) articles. It was found that the reporting of postoperative pain management in the studies was remarkably low, reflecting either under-reporting or under-use. Analgesic description, when given, was frequently too limited to enable reproducibility. Development of a

Why is it taking so long to improve reproducibility?

Berti & Cima 1955, quoted in Öbrink and Rehbinder



Hurni 1969, quoted in Öbrink and Reh binder

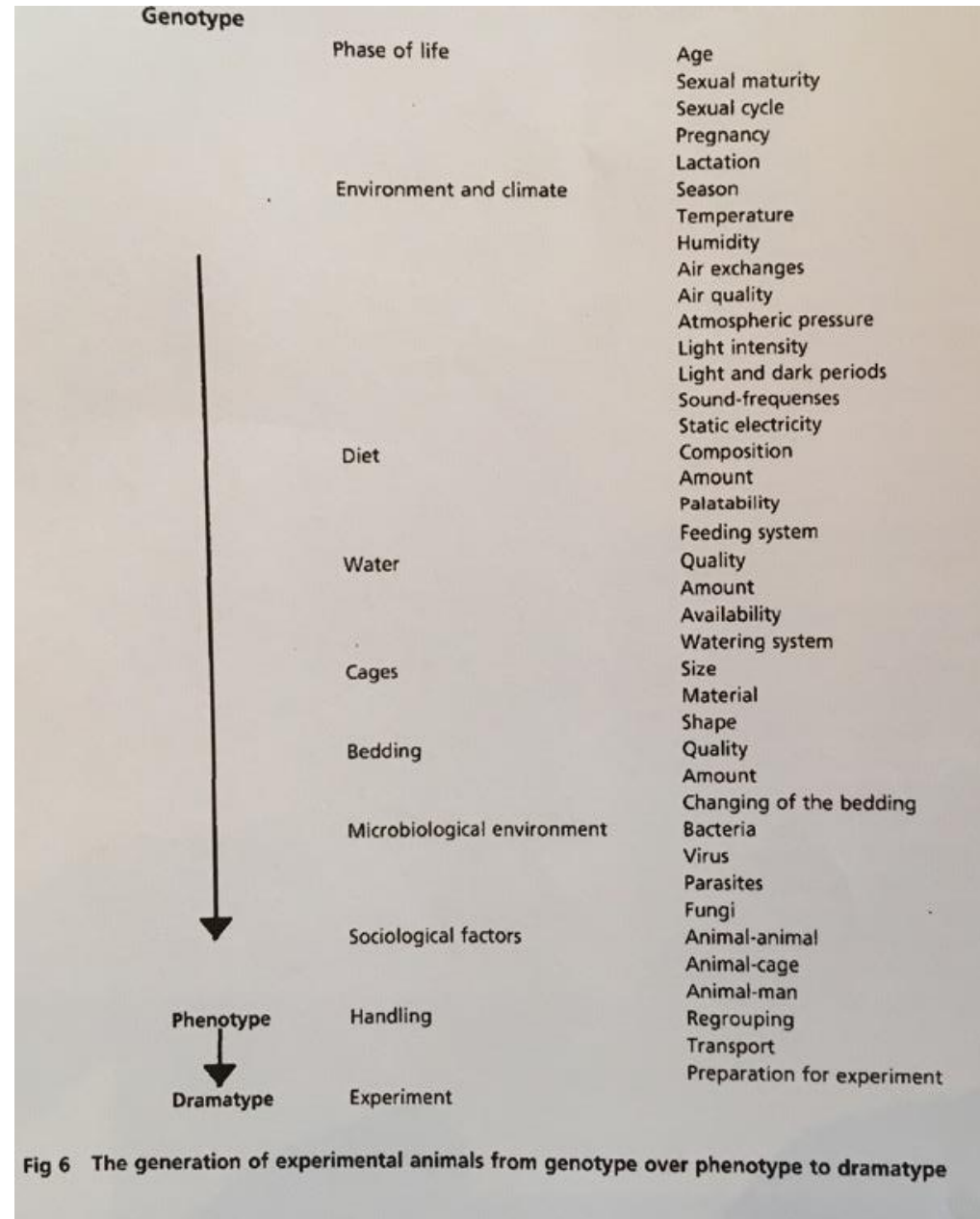


Fig 6 The generation of experimental animals from genotype over phenotype to dramatype

In designing an experiment, some of the areas which can be neglected ...

- poor literature searches
- lack of humane endpoints
- poor experimental design
- vague distribution of work and costs between the scientists and the animal facility
- insufficient evaluation of the facility's competence and infrastructure
- too little attention to transport and acclimation
- ignoring health risks for all involved
- lack of standard procedures for necropsy
- poor planning of waste disposal
- little discussion about the fate of the animals



PREPARE

<https://www.bls.gov/ooh/images/3077.jpg>

ARRIVE



<https://www.dreamstime.com>

PREPARE

- PREPARE (***P**lanning **R**esearch and **E**xperimental **P**rocedures on **A**nimals: **R**ecommendations for **E**xcellence*)

PREPARE: guidelines for planning animal research and testing

Adrian J Smith¹, R Eddie Clutton², Elliot Lilley³,
Kristine E Aa Hansen⁴ and Trond Brattelid⁵

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Abstract

There is widespread concern about the quality, reproducibility and translatability of studies involving research animals. Although there are a number of reporting guidelines available, there is very little overarching guidance on how to *plan* animal experiments, despite the fact that this is the logical place to start ensuring quality. In this paper we present the PREPARE guidelines: Planning Research and Experimental Procedures on Animals: Recommendations for Excellence. PREPARE covers the three broad areas which determine the quality of the preparation for animal studies: formulation, dialogue between scientists and the animal facility, and quality control of the various components in the study. Some topics overlap and the PREPARE checklist should be adapted to suit specific needs, for example in field research. Advice on use of the checklist is available on the Norecopa website, with links to guidelines for animal research and testing, at <https://norecopa.no/PREPARE>.

Keywords

guidelines, planning, design, animal experiments, animal research

Date received: 5 April 2017; accepted: 27 June 2017

Introduction

The quality of animal-based studies is under increasing scrutiny, for good scientific and ethical reasons. Studies of papers reporting animal experiments have revealed alarming deficiencies in the information provided,^{1,2} even after the production and journal endorsement of reporting guidelines.³ There is also widespread concern about the lack of reproducibility and translatability of laboratory animal research.^{4–7} This can, for example, contribute towards the failure of drugs when they enter human trials.⁸ These issues come in addition to other concerns, not unique to animal research, about publication bias, which tends to favour the reporting of positive results and can lead to the acceptance of claims as

in our experience, often underestimated by scientists. Even small practical details can cause omissions or artefacts that can ruin experiments which in all other respects have been well-designed, and generate health risks for all involved. There is therefore, in our opinion, an urgent need for detailed but overarching guidelines for researchers on how to plan animal experiments which are safe and scientifically sound, address animal

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The **PREPARE** Guidelines Checklist

Planning **R**esearch and **E**xperimental **P**rocedures on **A**nimals: **R**ecommendations for **E**xcellence

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PREPARE¹ intende presentare linee guida di pianificazione complementari a quelle di reportistica, quali quelle contenute in ARRIVE². PREPARE copre le tre maggiori aree che determinano la qualità della preparazione degli studi con gli animali:

- 1. Formulazione dello studio**
- 2. Dialogo tra ricercatori ed esperti dell'utilizzo di animali nella ricerca**
- 3. Controllo di qualità delle componenti dello studio**

Gli argomenti non verranno sempre affrontati nell'ordine in cui vengono presentati nel presente opuscolo, e alcuni argomenti si sovrappongono. La lista di controllo PREPARE può essere adattata per soddisfare esigenze particolari, come, ad esempio, gli studi in campo. Le linee guida PREPARE sono progettate per essere applicabili alla gestione delle strutture che ospitano animali, in quanto la loro qualità influenza significativamente gli esperimenti che vi si conducono. La versione completa di queste linee guida è disponibile sul sito web di Norecopa, sul quale si trovano anche link alle risorse globali, all'indirizzo <https://norecopa.no/PREPARE>.

Le linee guida PREPARE costituiscono un insieme dinamico destinato ad evolversi in quanto verranno emesse ulteriori linee guida specifiche per ogni specie animale e per situazioni particolari, come richiesto per il progredire dalle attività di eccellenza nel campo della scienza degli animali da laboratorio.

PREPARE:

Planning Research and Experimental Procedures on Animals: Recommendations for Excellence

15 argomenti

Formulare lo studio

1. Ricerca della letteratura
2. Questioni legali
3. Problemi etici, analisi del rapporto danno/beneficio e degli endpoint umanitari
4. Disegno sperimentale e analisi statistica

Dialogo tra ricercatori ed esperti dell'utilizzo di animali nella ricerca

5. Obiettivi e tempistiche, finanziamento e suddivisione del lavoro
6. Valutazione delle strutture
7. Istruzione e Formazione
8. Rischi sanitari, smaltimento dei rifiuti e decontaminazione

Controllo di qualità delle componenti dello studio

9. Sostanze oggetto di prova e procedure operative
10. Animali destinati alla ricerca scientifica
11. Quarantena e monitoraggio sanitario
12. Stabulazione e allevamento
13. Procedure sperimentali
14. Soppressione umanitaria, rilascio o reinserimento
15. Autopsia

checklist



ARRIVE:



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

Linee guida ARRIVE 2.0

La ricerca sugli animali: relazioni scientifiche
di esperimenti *in vivo*

Le linee guida ARRIVE 2.0 riassumono le informazioni da includere nelle pubblicazioni che descrivono la ricerca sugli animali e sono state pubblicate su *PLOS Biology* nel luglio 2020. Assicurano che le relazioni scientifiche degli studi siano sufficientemente dettagliate da arricchire la relativa base di conoscenze. Tale trasparenza permette ai lettori e ai revisori di esaminare la ricerca in modo adeguato, di valutarne il rigore metodologico e di riprodurre i metodi o i risultati.

Traduzione in italiano

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checklist



Why are the 3Rs important?

- they are now part of the legislation to protect animals and improve science quality
- they encourage discussion while a study which appears to need animals is being planned
- they are a tool to achieve ethically defensible animal studies
- they advance the implementation of replacement techniques
- they increase public understanding of the need for animal research and testing



norecopa.no/norina/blood-collection-in-mice-using-the-saphenous-vein-an-alternative-to-retro-orbital-collection

Statistics in medical research: Common mistakes:

doi: [10.1016/j.jtumed.2023.04.004](https://doi.org/10.1016/j.jtumed.2023.04.004)

- **1. Sampling bias.** This occurs when the sample of study participants is not representative of the population being studied, and can lead to inaccurate conclusions relating to the effects of a particular intervention in that population or the inaccurate generalizability of the results.
- **2. Inappropriate sample-size calculation.** The validity of a study would be negatively affected if the calculated sample size was insufficient or incorrectly calculated.
- **3. Effect of confounding variables.** These are variables that are related to both the exposure and the outcome being studied and can lead to false associations.
- **4. Errors in the application of statistical tests.** Choosing the wrong test for a particular dataset affects the validation of observations during research.
- **5. Type I and Type II errors.** Type I errors occur when a study incorrectly concludes that there is a significant effect when there is not (erroneously finding a difference). Type II errors occur when a study incorrectly concludes that there is no significant effect when there is (the inability to identify a real difference).
- **6. Failure to adjust for multiple comparisons.** When multiple comparisons are performed in a study, the probability of a Type I error increases.
- **7. Inappropriate use of p-values.** p-Values are often misinterpreted as a measure of effect size or clinical relevance, when in fact they only indicate the probability of observing the study results by chance.
- **8. Data dredging or "fishing".** This occurs when multiple analyses are conducted on a dataset to identify significant associations, without a clear hypothesis or theoretical framework.
- **9. Publication bias.** This occurs when studies with positive or statistically significant results are more likely to be published than those with negative or non-significant results, thus leading to overestimation of the true effect size

... Beyond the 3Rs, Validity

The 3Vs: construct validity, internal validity and external validity (Hanno Würbel)

Aimed at questioning and improving the scientific validity of animal and non animal models

- Construct validity** (*Pose research question and construct a suitable experiment*): am I measuring the correct variables?
- Internal validity** (*The extent to which the design and conduct of the trial eliminate the possibility of bias*) : are my experiments biased (selection bias, control bias etc)?
- External Validity** (*Generalizability*): Are my results generalizable outside my lab and/or translatable?

- The 3Ss: Good Science, Good Sense, Good Sensibilities (Carol Newton)
Ensuring that common sense and critical anthropomorphism are applied to science
- The 7Rs: 3Rs + responsible, robust, rigorous, reproducible

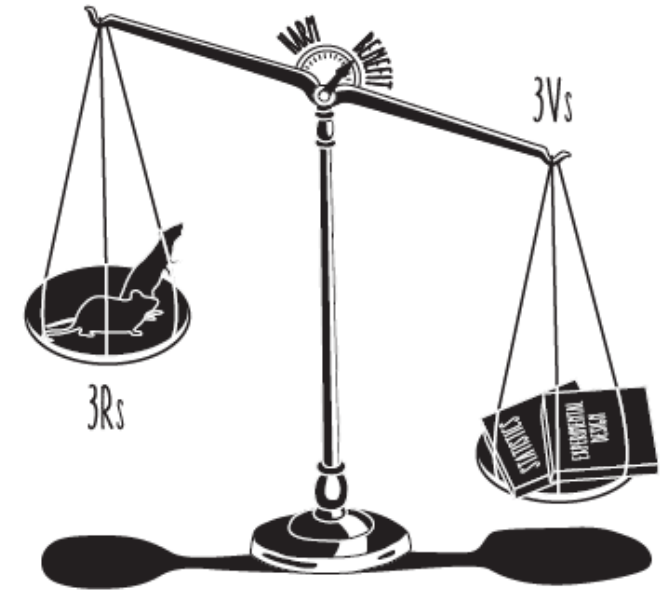


FIGURE 1 | Refined procedure for harm-benefit-analysis (HBA) in animal research. Whereas 3Rs methods minimize the weight of harms to the animals on the HBA balance, methods to improve the scientific validity of the research (3Vs) maximize the value of study outcomes, thereby facilitating the expected benefits.

Essential reading

- Points of significance, Articoli di Martin Krzywinski & Naomi Altman in Nat. Neuroscience
 - Designing comparative experiments
 - Power and sample size: The ability to detect experimental effects is undermined in studies that lack power.
 - Interpreting P values: A P value measures a sample's compatibility with a hypothesis, not the truth of the hypothesis.

Documenti

- Newsletter Centro 3R
<https://www.centro3r.it/sites/default/files/Newsletter%20Centro3RJune2023.pdf>
- Prepare e arrive (in risorse, sito Centro 3R)
- https://www.centro3r.it/sites/default/files/prepare_checklist_italian.pdf
- <https://www.centro3r.it/it/linee-guida-arrive>

- <https://forms.gle/LVmygXLwPqAbdCU26>



Sondaggio Centro 3R

Lo scopo del questionario e' di valutare l'effetto degli sforzi fatti dal Centro 3R per inserire le tematiche 3R nei percorsi formativi e guidare le future attività.



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- Grazie 😊

- Alcune slides sono state fornite da Adrian Smith di Norecopa
<https://norecopa.no/>

