Ana Sofia Rodrigues Machado - Champalimaud Foundation

Report

Workshop on Teaching Experimental Design for Animal Experiments
September 12 -13 Sep 2022
Faculty of Medicine, Porto, Portugal

During these 2 days of the workshop, we exchange ideas on the content of courses. We shared teaching material and discussed different ways of lecturing. This workshop follows the rationale of the FELASA Experimental Design Working group. The goal is to develop a common education and training framework to fulfill the requirements under the **Directive 2010/63/EU** on the protection of animals used for scientific purposes.

Day 1

***Thomas Steckler**
Issues in Experimental Design that Need Addressing

The problem: Low/Non-reproducibility studies are caused by two sorts of failure: false-negative and false-positive results. False-negative is related to variation across animals and a small sample size. While false-positive is related to bias or other errors in the conduct of the experiment. The cost of low/non-reproducible studies is around billions of dollars annually in the USA!

Explained what variability is. The causes and how to reduce it. The importance of using genetically defined animals (ex, Isogenic strains, genetically modified strains). The implication of stress, social interaction, enriched environment, and habituation. Check the overall health of the animal. Identify bias and design defaults in different types of experiments. How to avoid it? Randomisation and blind trial.
Identify the experimental unit and recognise pseudo-replication.
What is affecting significance? Significance is the ratio between signal and noise. Importance of reducing noise/variation. Meaning of P-values and the power of the experiment. The power should be between 80% and 90% and it does not increase linearly with an increase in sample size.
Calculate and determine the sample size using power analysis or the resource equation method.

***Derek Fry and Manuel Berdoy**
Some experimental design variations

Factorial approach:
Use this to reduce the number of animals used in the experiment.
'Factor' is a discrete variable - what we can control: treatment, gender, age…
It has at least two fixed effect factors (ex; gender: female or male; treatment: A, B, C).
Goal:
*Investigate the effect of each factor on the dependent variable, independently.

*Look for interactions across factors.
*Using the same number of animals, we can obtain more knowledge.

Randomize block design:
The experiment is divided into blocks. We can investigate “n” treatments in each block, which means “n” experimental units. Each block is separately randomised. It can be separated in space and/or time.
Advantages: increase the power and the repeatability of an experiment
Most of these experimental designs come from the studies of R. A. Fisher in agriculture.

The results of an experiment needed to be statistically analysed.
Use the analysis of variance (ANOVA) for any number of treatment groups.
The results are presented in a single table showing both the experiment’s structure and the relevant results.

***Carlos Sorzano**
Approaches to teaching statistics

Several materials to teach statistics were shared,
Define important concepts through examples (in some cases link the examples with the simplest mathematical formulas, like sample size),
Time spent on each topic,
Promote discussions rather than lectures.
Use videos but only 10-20%.
Main message: “Control what you can, block what you cannot, and randomize the rest”

Unfortunately, things are not so simple! It involves sophisticated concepts; information is too spread or too technical. We end up using a t-test!
Typical courses for practitioners are only 2-50h? and in contrast, it takes a huge amount of time to master!
Ended with Open Questions to reflect on ways how to improve this course.

Activities and group discussion:

1- Pre-Quiz: Before the presentations, all the participants respond to a quiz unanimously

All the participants were divided into 5 groups

Exercise 1:
*Identify design faults in different types of experiments
*Identify the experimental unit in different basic experimental arrangements
*Recognise pseudoreplication in a simple erroneous experimental arrangement

Exercise 2:
*Decide which of the following designs is the most efficient and suitable:

a) 2 or more groups with experimental units randomly allocated to each group
b) Factorial design
c) Block design

Exercise 3:
*Come up with experimental scenarios for group discussion

Comments and discussion on the approaches and material shown so far

Day 2

Discussion of the scenarios produced by the groups:
Our group (group 4) proposed the following scenario:

Original
A new treatment needs to be tested for its plasma concentration and cytokine response dynamics. The vehicle and two doses of treatment (high and low) will be administered intravenously to six single-housed male beagle dogs with a period of 10 days in between the dosages (i.e., the known washout period of the drug). Each of the animals will receive all 3 treatment settings once in a sequence-randomized manner, and a blood sample will be taken every 24 h post-administration to follow up the drug concentration and cytokine responses over time.

What kind of design is it? What is the experimental unit?
A sequence-randomized crossover design (animal as a block).

The following texts were proposed by Derek Fry. As an example of a better scenario.
Suggested text for quiz
To determine the cytokine effect of a new drug, researchers wish to compare high and low doses of the agent against vehicle control using singly-housed beagles. Plasma cytokine levels 1day after drug or vehicle injection will be measured. A pilot study has shown that there will be no drug left in a beagle 10 days after injection.

What would be the most efficient design (using the fewest dogs) for this experiment?
A – A completely randomised design
B – A cross-over experiment
C – A factorial experiment
D – A randomised block experiment
E – I am not sure.

Suggested text for group discussion
Researchers wish to determine the variation of plasma levels and cytokine response with time after injection of a new drug. They propose to compare high and low doses of the agent against

vehicle control using singly-housed beagles. Blood samples will be taken to determine plasma levels every 24h after drug or vehicle injection for 6 days. A pilot study has shown that there will be no drug left in a beagle 10 days after injection.
How should they plan the experiment?

Useful discussion points
Randomisation of the beagles to the sequence of injections (and therefore sampling) for each treatment.
Use of a single person for all the injections and a single person for all the sampling, or randomising the people used.
Use of both sexes
How to determine the numbers needed

*When writing a scenario, the main goals are:
1. a clear statement of purpose
2. text giving the proposed comparisons and a general description of the procedure involved
3. a statement of the outcome measure(s)

*Next, we had short presentations by participants of other approaches found effective:

Summary:
The presentation should be simple with an output clear and less text
Use more visualization: Schematics and Figures
Use software to calculate sample size, use graphics to show the effect of power
EDA - the experimental design assistant
Bring student projects as an example of a case study, and swap projects between students.

We discuss “Flipping” and the use of pre-course and online material

Post-quiz using some of the scenarios discussed during the group activities. The group improved the result when compared to the pre-quiz.

Discussion and recommendation of the learning outcomes for day 1 of a 2-day course.
Examples of approach and material used at the Bologna workshop. Attached is our recommendation - group 4

<u>Final Remark</u>
Students should:
Recognise causes of biological variability.
Identify the best design experiment.
Use appropriate statistical methods.
Ensure consistency between experiments.
Look for expert advice.