Check-in Code:

Edinburgh Napier UNIVERSITY

Research Skills

Week 3
The Scientific Method
&
Experimental design
Part 2

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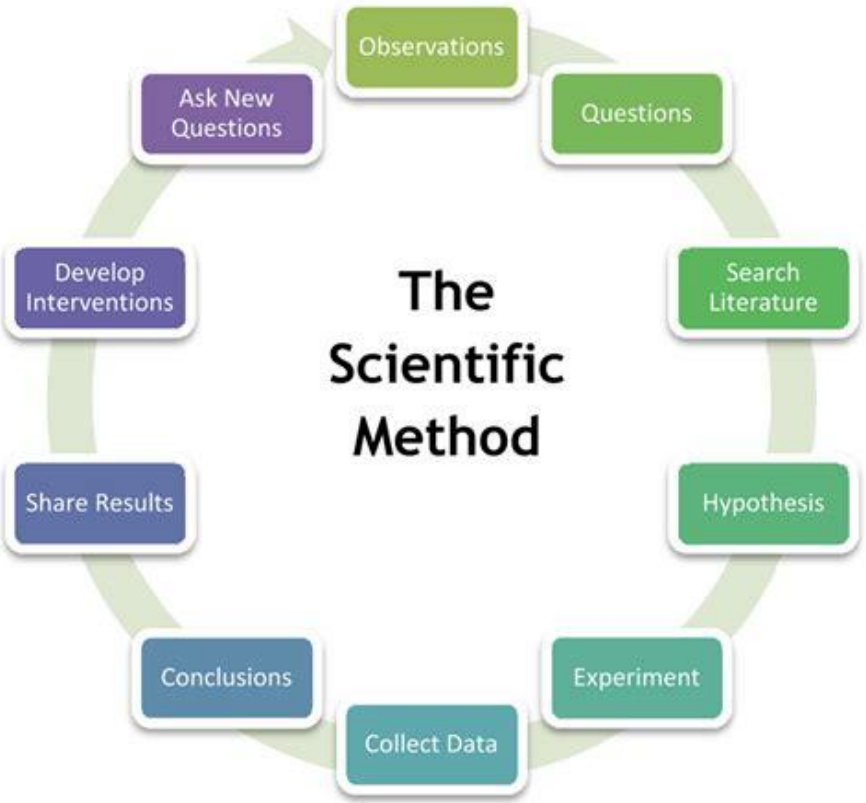


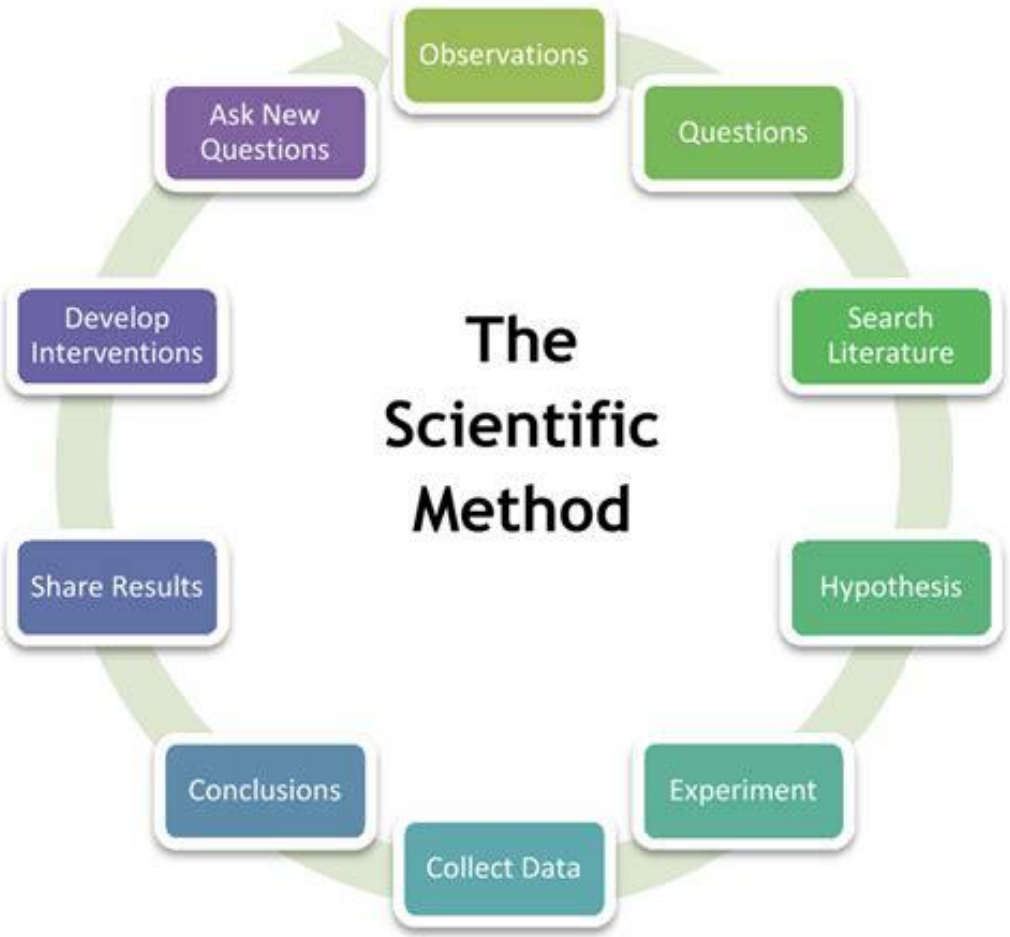
LOs for Today's lecture

In this lecture, we will cover:

- How to set up a controlled empirical experiment
- Basic principles of research models
- How to ensure unbiased samples/sampling
- Confounding variables
- Replicates and pseudoreplicates
- Sample size and type I and type II errors

Where do we start?

1. An observation that prompts you to ask a question.
2. Forming a hypothesis based on the question/observations
3. Testing the hypothesis by designing one or more experiments
4. Analysing the results
5. Interpreting and evaluating the results. Can you prove or disprove your hypothesis? Or do you need to modify the experiment, look at a new hypothesis etc? if so, go back to step 2…
6. Draw conclusions



A good experiment should be repeatable by others, and should be designed to directly test the hypothesis, whilst minimising bias, errors and confounding variables

Summaring from last week…

1. An experiment is a **<u>controlled</u> test of a hypothesis** by gathering data under controlled conditions.
2. The goal is to be able to accept or reject the hypothesis

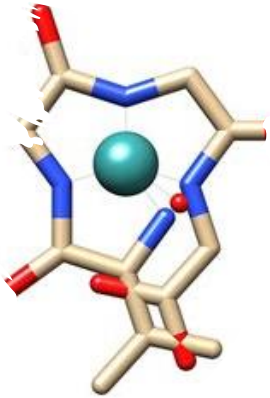
A good Experiment

Should:

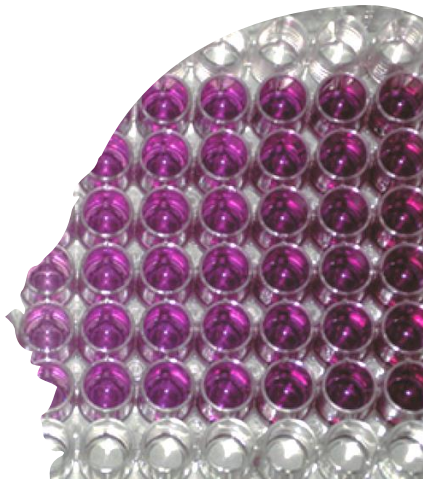
- Use an appropriate model and method
- Be controlled for everything other than the variable(s) tested
- Use unbiased selection and testing
- Use a (sufficiently) large number of samples
- Be repeated/repeatable

Experimental models

- The experiment must address the research problem: what parameter is tested in the experiment?
- Ideally, you should be able to establish direct causality: ‘If X then Y’. Association does not prove cause and effect
- What do you compare your results to? E.g:



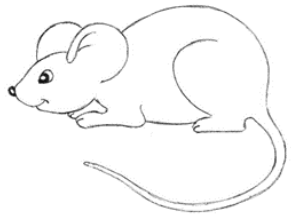
- Untreated vs treated sample/subject
- A ‘gold standard’ or sample with known properties
- X treatment vs Y treatment



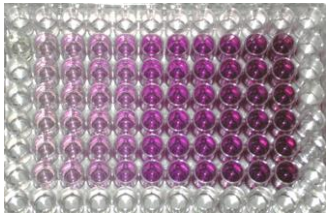
Experimental models

'Make everything as simple as possible, but no simpler'
Albert Einstein

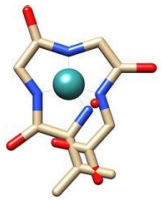
- The variables that can impact your experiment depend on your model
- You must consider potential variability/errors in each very step of your experiment:



- Samples/sampling → Unbiased sampling
- Methods → Assay controls



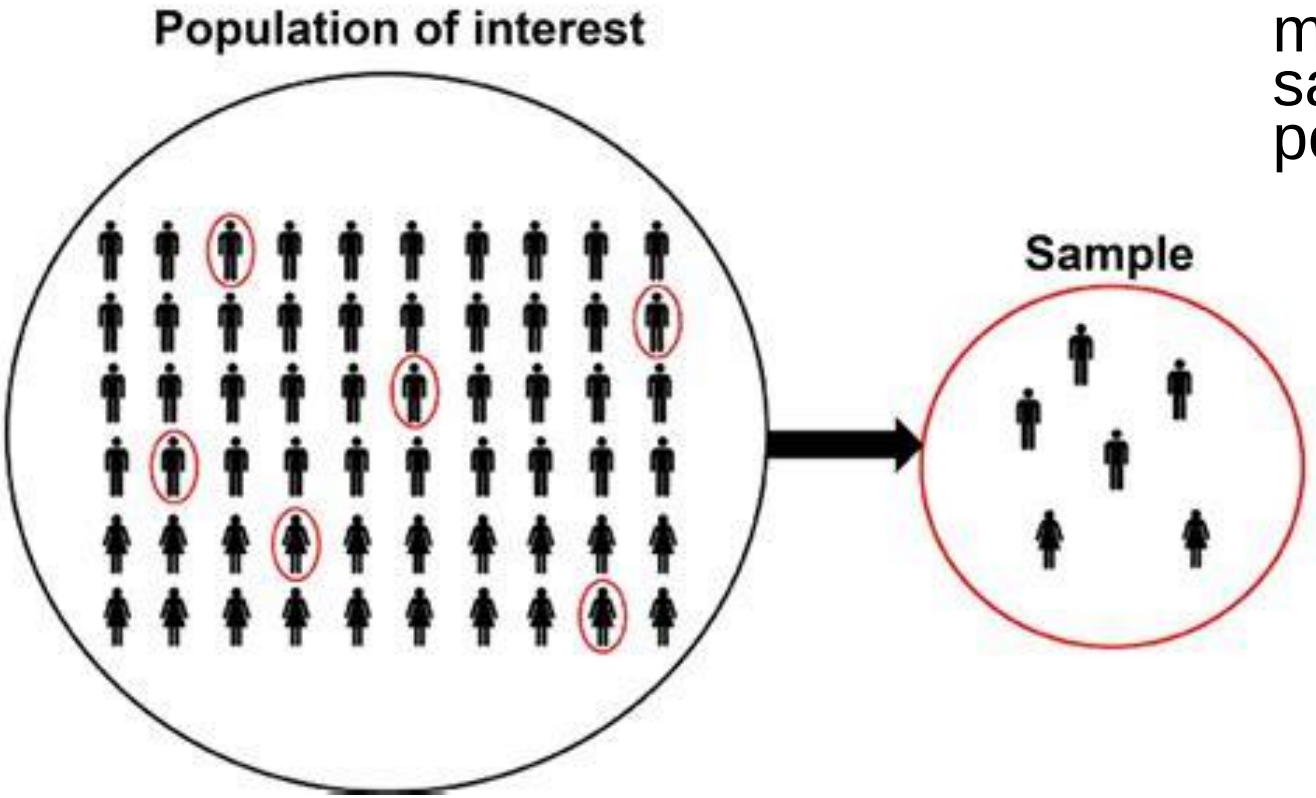
- Environment → Control external factors
- Analysis → Stats, interpretation



Increasing complexity

Samples and Sampling

You can rarely empirically test an entire population, or all aspects of a phenomenon

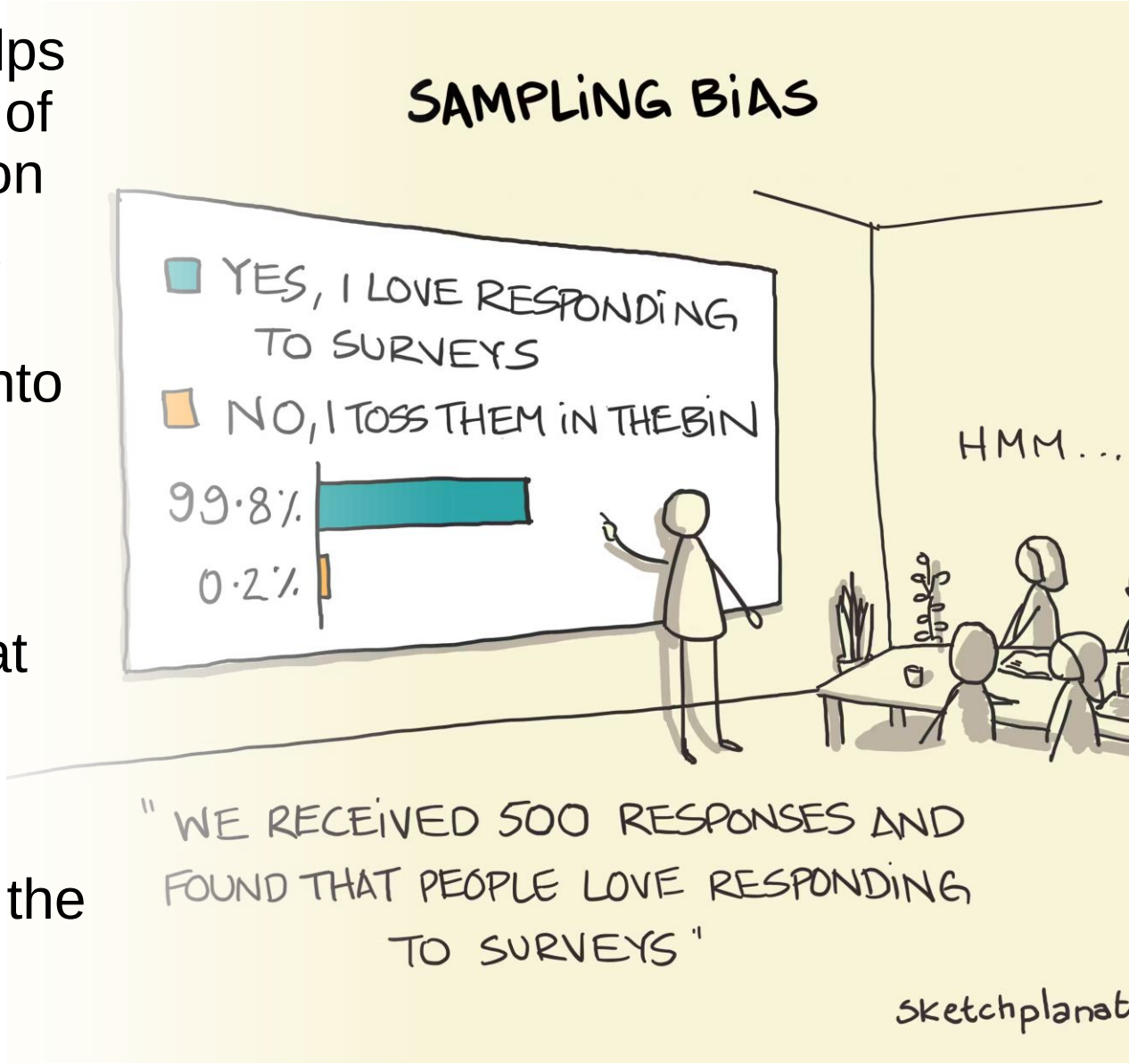


Representativeness

- Are the study subjects/cells/samples representative of the population you want to study?
- Ideally, your study material is a random sample from the population of interest.
  - Also: does your model replicate the research problem accurately?

Sample selection should be unbiased

- **Randomisation** helps to reduce the effect of uncontrolled variation
- **Stratification** is the process of dividing subjects/ samples into homogeneous subgroups before sampling.
- **Blinding** means that analysis should be made without knowledge of what experimental group the sample comes from



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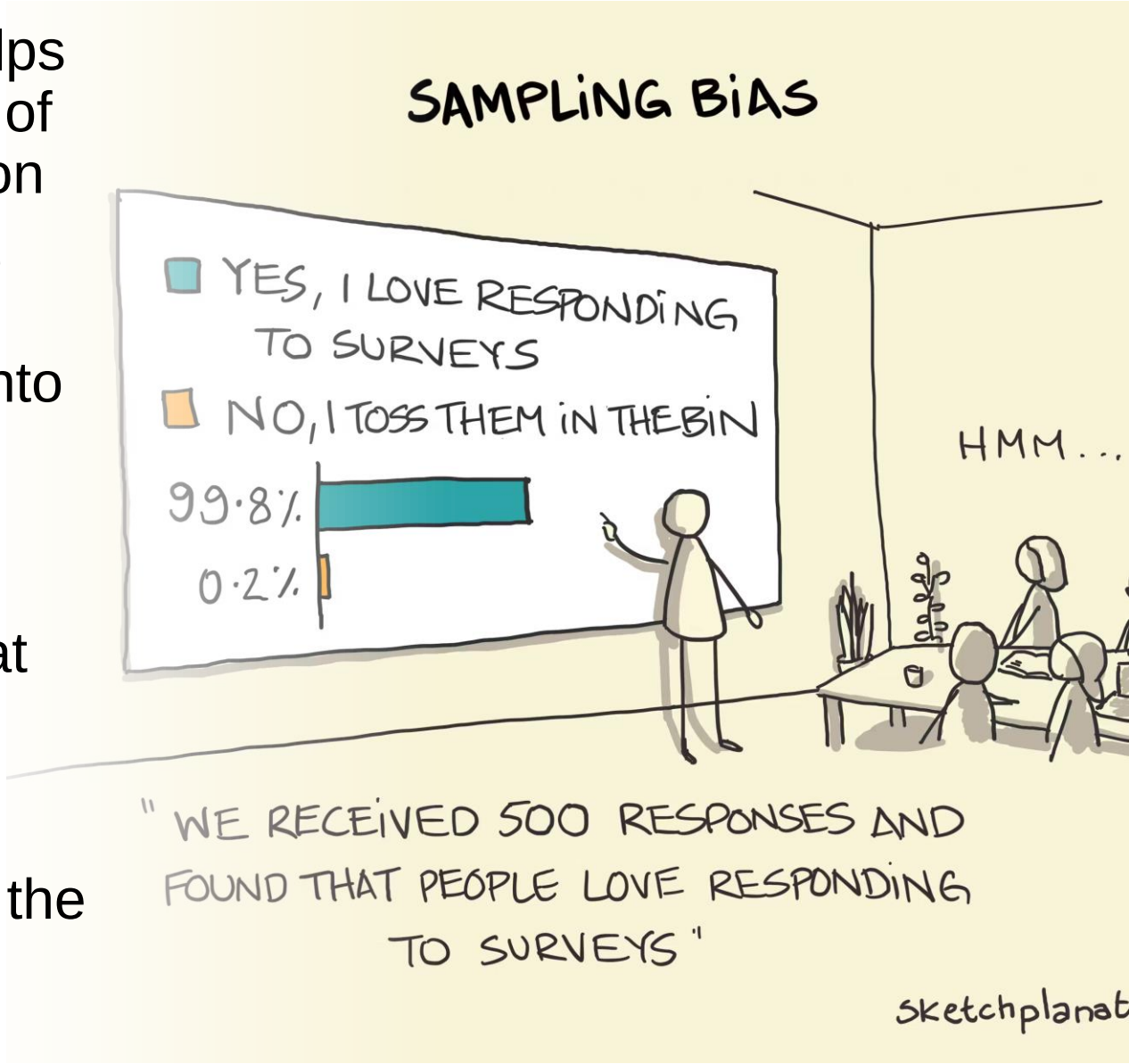


Confounding Variables

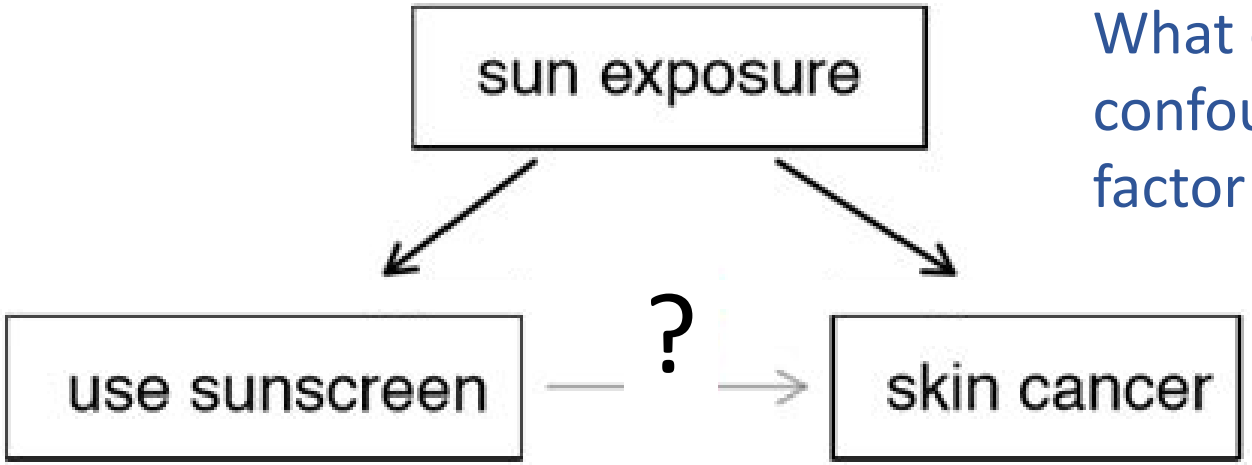
A variable that correlates with the independent **and** the dependent variable

Example

Hypothesis: 'Sunscreen causes skin cancer'

→Perform study tracking sunscreen use and skin cancer

Finding: The more sunscreen a person uses, the more likely they are to having skin cancer

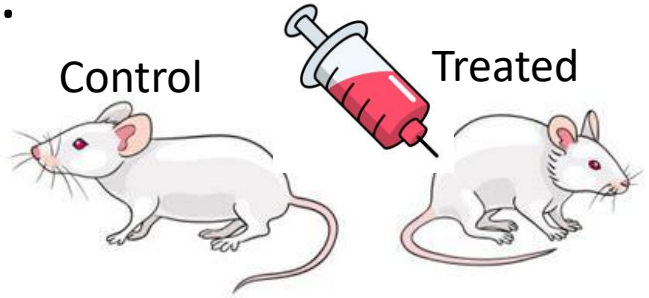


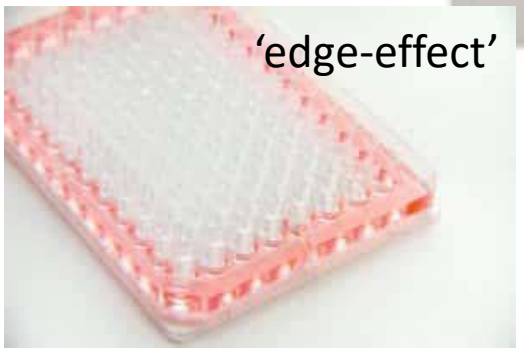
What could a confounding factor be here?

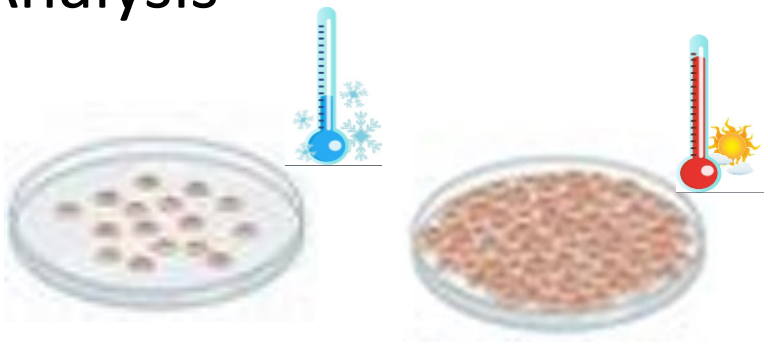
Controlling confounding Variables

Confounding variables (and other sources of error) may be present in any part of your experiment…

- Samples: selecting & processing
- Administering treatment
- Models: e.g genetic knock-out model that has multiple and diverse effects on the animal
- Methods, protocols
- Environment
- Analysis







Ways of dealing with confounding variables

- **Randomisation**
- **Stratification**
- **Restriction** ⟶ *Can be implemented far more comprehensively in cell- or animal studies*
- **Matching**
- **Use of appropriate controls** (more on controls in week 4)
- **Standardised methods**
- **Validates equipment**

Replicates vs pseudo-replicates

‘ A good experiment should be repeated/repeatable’

Pseudo-replicates
(technical replicates)

- Replication of experimental samples that are not independent from one another.
- Examples:

Cells from one cell culture dish are plated into 3 parallel wells on a 96-well plate for a cell viability assay.

- A drug sample is divided into 3 vials and the concentration of the drug sample is tested in all 3 vials.

Assay repeats (‘biological’ or ‘genuine’ replicates)

- Replication of experimental samples that are independent from each other
- Example:

A drug tested in two different individuals

A cell viability test using cells from different cell culture dishes, preferentially on different days/different cell passage (number of cell divisions)

Example: pseudo-replicate vs genuine replicate

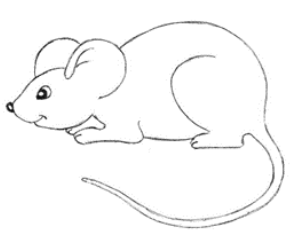
BSA Protein Assay

Protein standard at 6 concentrations

Protein sample, 4 dilutions

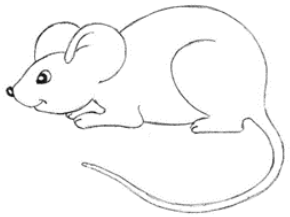
Mouse 1

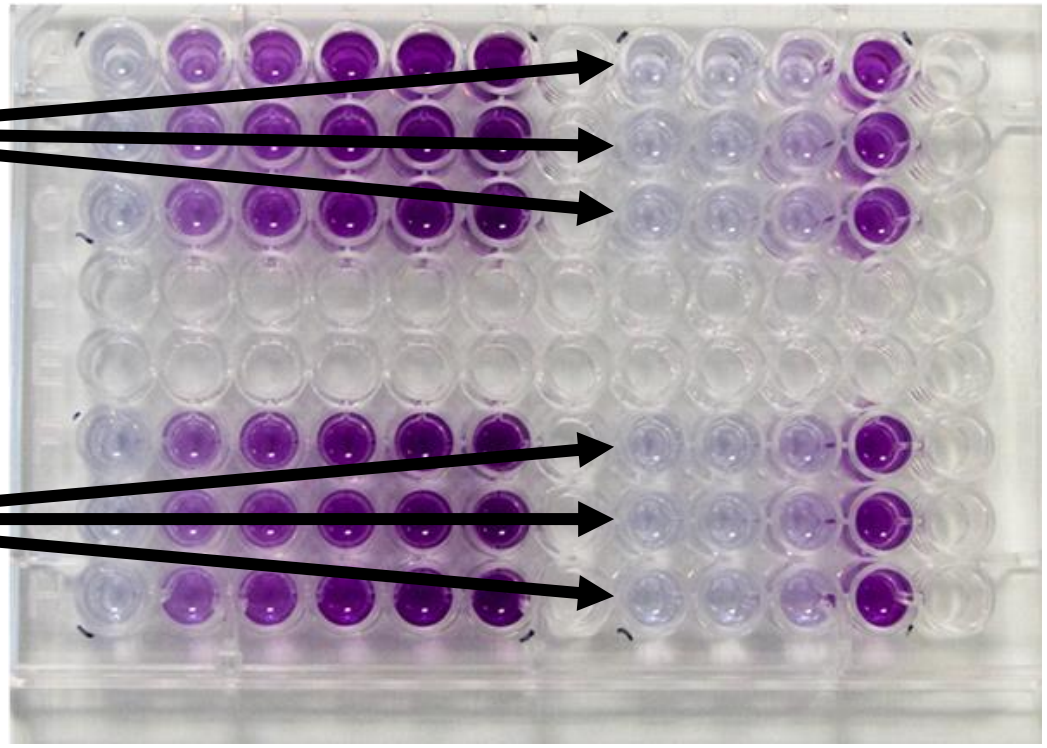
Protein sample 1



Mouse 2

Protein sample 2

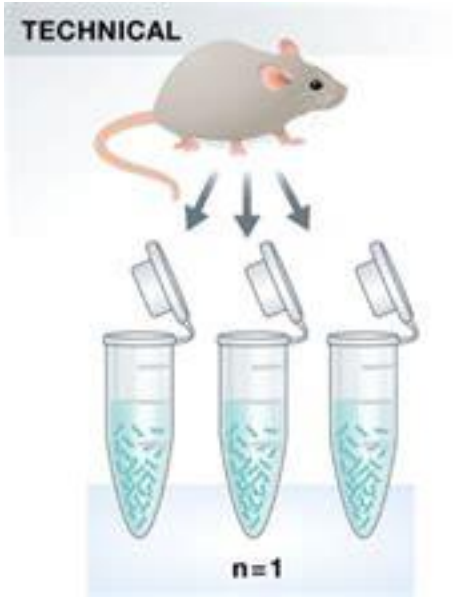


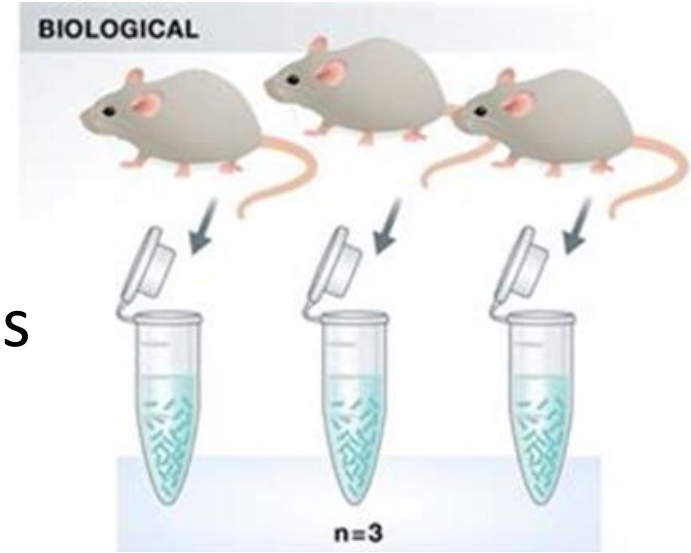


How many pseudoreplicates and real replicates are carried out here?

Real and pseudoreplicates

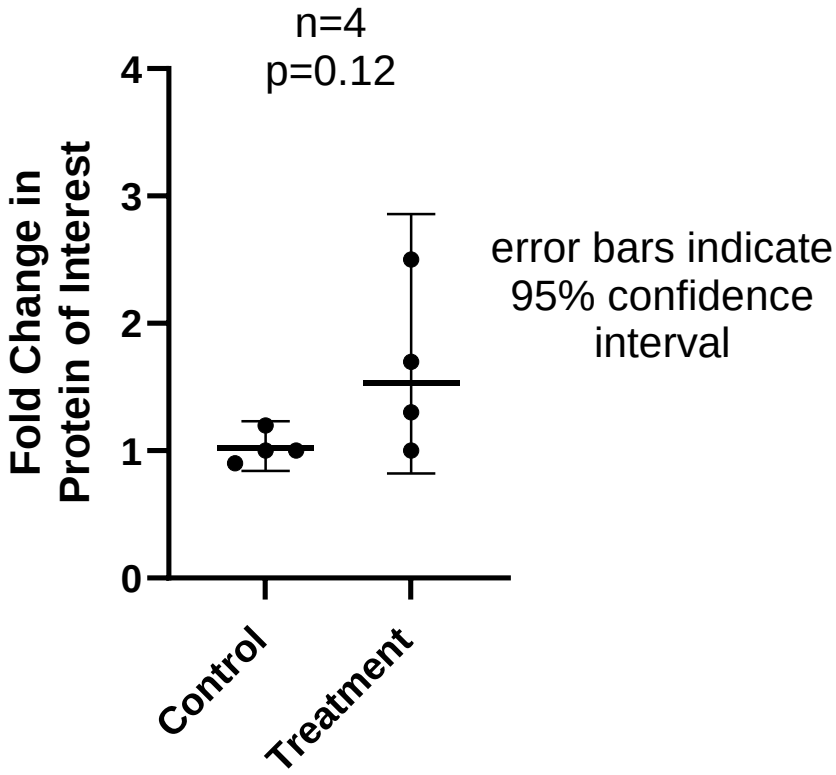
- Pseudoreplicates are used to increase and precision – safeguard against e.g. pipetting inaccuracies.
- Pseudoreplicates should not be considered as separate samples → usually a median or mean value is calculated for pseudoreplicates
- Only real replicates will capture biological variability between samples or individuals
- Counting pseudoreplicates as separate samples can lead to serious errors in your statistical analysis.





Determining sample size

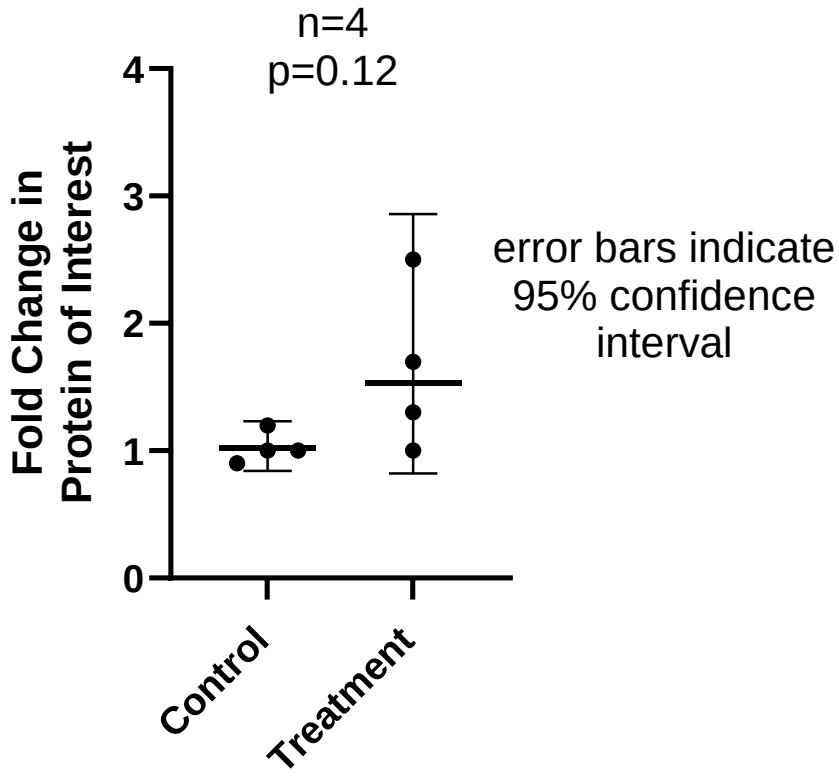
- Let's say you want to find **if a drug treatment changes the expression levels of your protein** of interest
- You set up an experiment using **4 control samples and 4 treated samples**
- Then you **measure the protein level** in all samples and **calculate the fold-change** in protein levels between control and treatment



Interpret these results

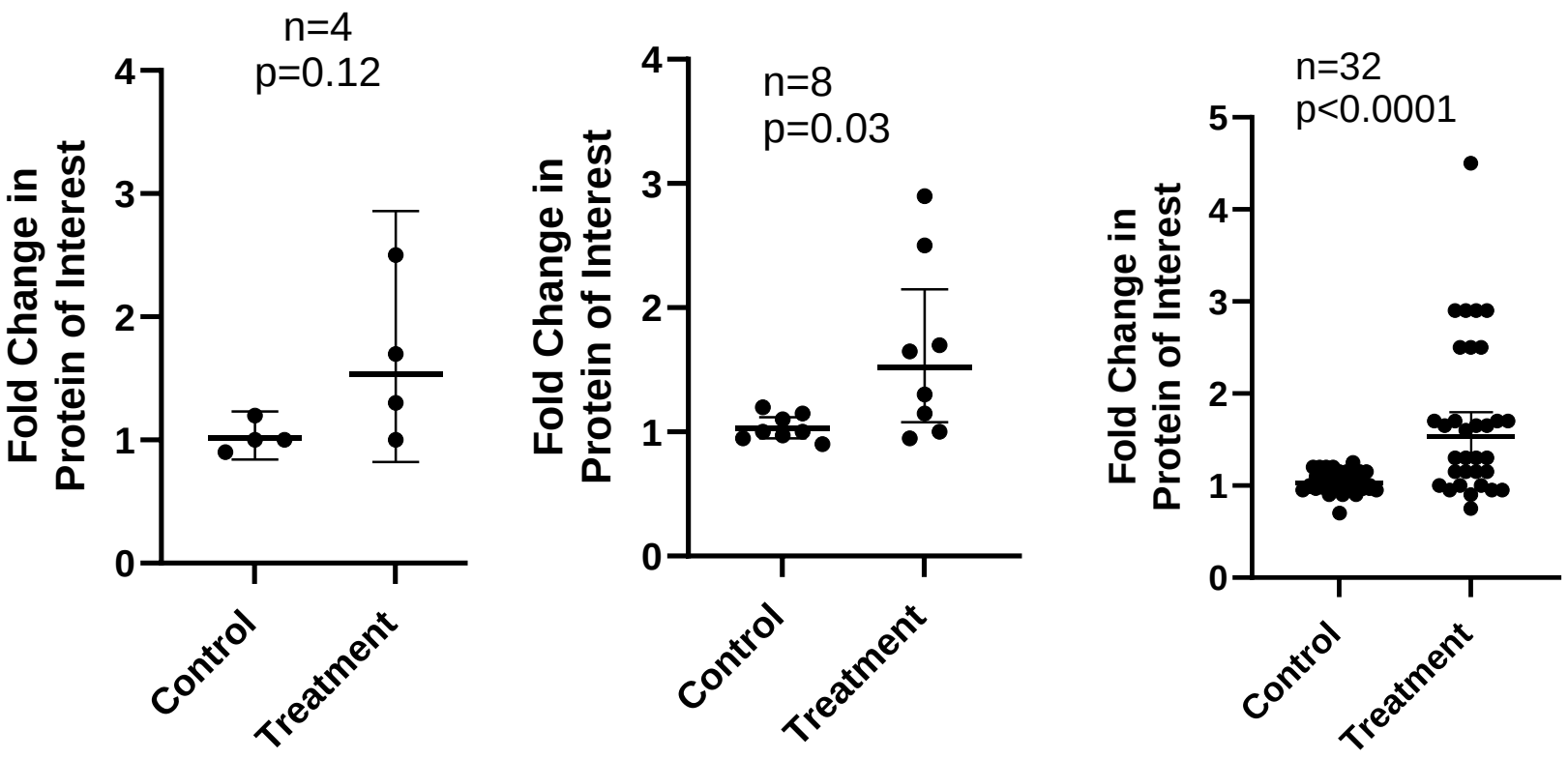
Type I and Type II errors

- In statistics a type I error means you make a false positive conclusion → you think the treatment has an effect but it actually has not
- A type II error is when you make a false negative conclusion → you think the treatment does not have an effect, but actually it does



What type of error could we have here?

Effect of sample size on your data



Higher sample size → more reliable estimation of the sample mean

If you know the effect size and level of variability, you can calculate how many samples you are needed for a reliable result = 'power calculation'

Sample size summary

- Be aware of type I and type II errors
- Examine your data distribution – are all results close together or are they highly variable? Do control and treatment group error bars overlap? If so, you may not have an effect…or you need more samples to see a significant effect!
- If your effect is small or rare, you will need a higher number of samples to determine an effect
- Especially in cases where your experiment is expensive, time-consuming or there are ethical considerations, it is important to estimate required sample size in the planning stage, before carrying out experiments.

Experimental error

No experiment is ever perfect…

- Human error
- Equipment error
- Experimental design error
- Incorrect analysis
- Environmental factors (e.g.temperature changes in the lab
- Factors out with the control of the experiments (e.g. powercut)

Questions?

Tutorial today at:

11-12 in SIG_2.D.07

or

12-13 in SIG_3D-07

'DISRUPTIVE' SCIENCE HAS DECLINED — EVEN AS PAPERS PROLIFERATE

The proportion of publications that send a field in a new direction has plummeted since the 1940s.

By Max Kozlov

The number of science and technology research papers published has skyrocketed over the past few decades — but the 'disruptiveness' of those papers has dropped, according to an analysis of how radically papers depart from the previous literature[1].

Data from millions of manuscripts show that, compared with mid-twentieth-century research, that done in the 2000s was much more likely to push science forward incrementally than to veer off in a new direction and render previous work obsolete. Analysis of patents from 1976 to 2010 showed the same trend.

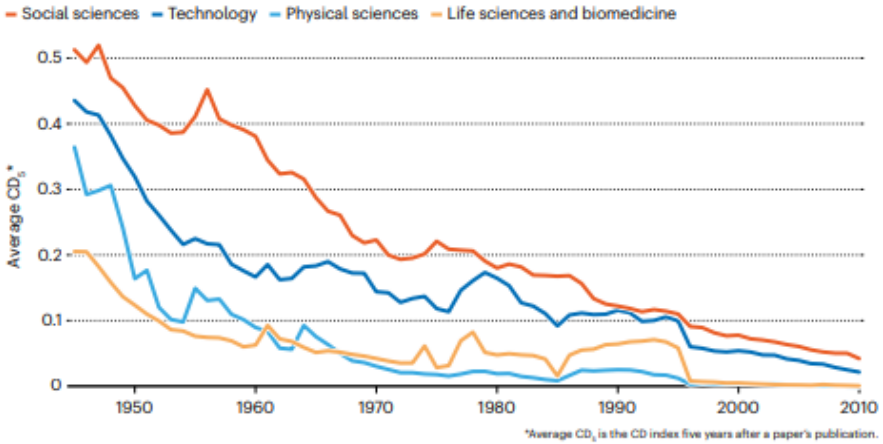
"The data suggest something is changing," says Russell Funk, a sociologist at the University of Minnesota in Minneapolis and a co-author of the analysis, which was published on 4 January in *Nature*. "You don't have quite the same intensity of breakthrough discoveries you once had."

Telltale citations

The authors reasoned that if a study was highly disruptive, subsequent research would be less likely to cite the study's references, and instead would cite the study itself. Using the citation data from 45 million manuscripts and 3.9 million patents, the researchers calculated a measure of disruptiveness, called the CD index,

DISRUPTIVE SCIENCE DWINDLES

To quantify how much a paper shakes up a field, researchers used a metric called a CD index, which ranges from 1 for the most disruptive papers to –1 for the least disruptive. Analysis of millions of papers shows that disruptiveness has fallen over time in all analysed fields.



*Average CD_5 is the CD index five years after a paper's publication.

It's great to see this [phenomenon] documented in such a meticulous manner," says Dashun Wang, a computational social scientist at Northwestern University in Evanston, Illinois, who studies disruptiveness in science. "They look at this in 100 different ways, and I find it very convincing overall."

Other research[2] has suggested that scientific innovation has slowed in recent decades, too, says Yian Yin, also a computational social scientist at Northwestern. But this study offers a "new start to a data-driven way to investigate how science changes", he adds.

Disruptiveness is not inherently good, and incremental science is not necessarily bad, says Wang. The first direct observation of gravitational waves, for example, was both revolutionary and the product of incremental science, he says.

The ideal is a healthy mix of incremental and disruptive research, says John Walsh, a specialist in science and technology policy at the Georgia Institute of Technology in Atlanta. "In a world where we're concerned with the validity of findings, it might be a good thing to have more replication and reproduction," he says.

Why the slide?

The drastic change might stem in part from changes in the scientific enterprise. For example, large research teams have become more common, and Wang and his colleagues have found[3] that big teams are more likely to produce incremental than disruptive science.

Finding an explanation for the decline won't be easy, Walsh says. Although the proportion of disruptive research dropped significantly between 1945 and 2010, the number of highly disruptive studies has remained about the same. The rate of decline is also puzzling: CD indices fell steeply from 1945 to 1970, then more gradually from the late 1990s to 2010. "Whatever explanation you have for disruptiveness dropping off, you need to also make sense of it levelling off" in the 2000s, he says.

1. Park, M., Leahey, E. & Funk, R. J. *Nature* **613**, 138–144 (2023).
2. Cowen, T. & Southwood, B. Preprint at SSRN http://doi.org/10.2139/ssrn.3822691 (2019).
3. Wu, L., Wang, D. & Evans, J. A. *Nature* **566**, 378–382 (2019).

Link to online paper:
https://www.nature.com/articles/d41586-022-04577-5

Additional Reading

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