Experimental Design

Basic principles

1. Questions/goal of the experiment.

2. Comparison/control.

3. Replication.

4. Randomization.

5. Stratification (aka blocking).

6. Factorial experiments.

Example

Question:

Does salted drinking water affect blood pressure (BP) in mice?

Experiment:

1. Provide a mouse with water containing 1% NaCl.
2. Wait 14 days.
3. Measure BP.

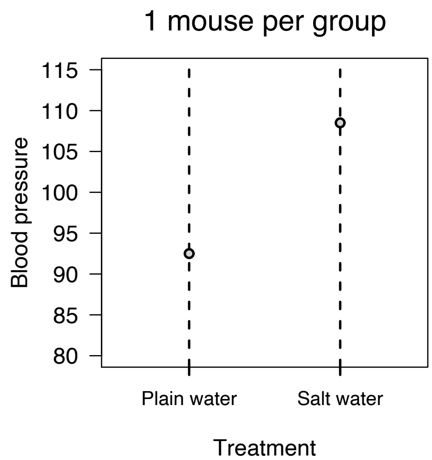
Comparison/control

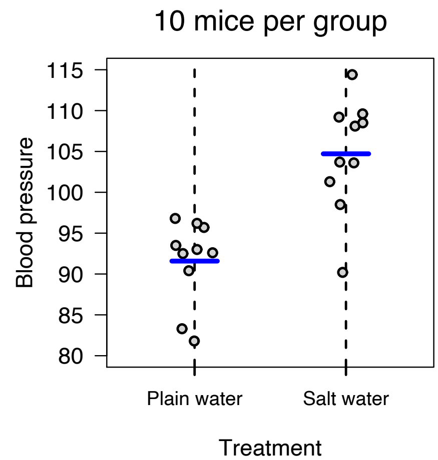
Good experiments are comparative!

- Compare BP in mice fed salt water to BP in mice fed plain water.
- Compare BP in strain A mice fed salt water to BP in strain B mice fed salt water.

$\longrightarrow$ Ideally, the experimental group is compared to concurrent controls (rather than to historical controls).

Replication



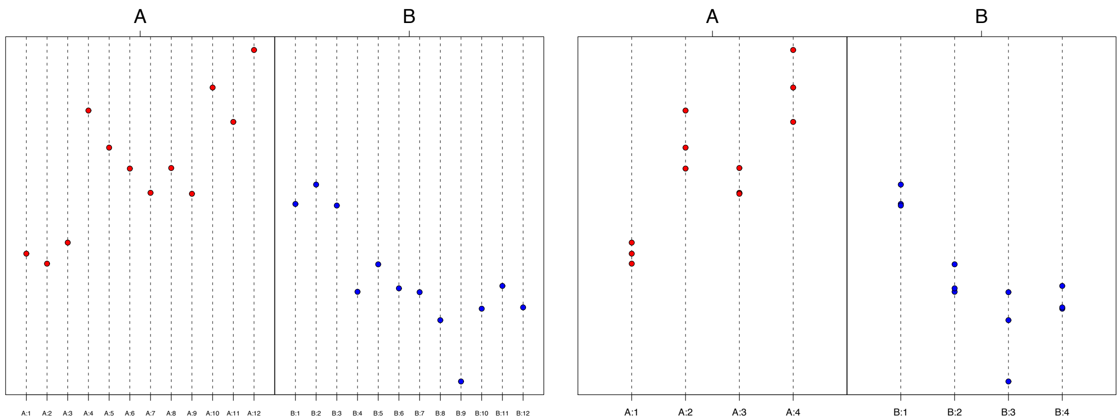


Why replicate?

- Reduce the effect of uncontrolled variation (i.e., increase precision).
- Quantify uncertainty.

⟶ An estimate is of no value without some statement of the uncertainty in the estimate!

Replication



Randomization

- Experimental subjects (“units”) should be assigned to treatment groups at random.
- At random does not mean haphazardly.
- One needs to explicitly randomize using a computer.

Why randomize?

- Avoid bias.

  For example: the first six mice you grab may have intrinsically higher BP.

- Control the role of chance.

  Randomization allows the later use of probability theory, and so gives a solid foundation for statistical analysis.

Stratification

- Suppose that some BP measurements will be made in the morning and some in the afternoon.

- If you anticipate a difference between morning and afternoon measurements:

  - Ensure that within each period, there are equal numbers of subjects in each treatment group.
  - Take account of the difference between periods in your analysis.

- This is sometimes called “blocking”.

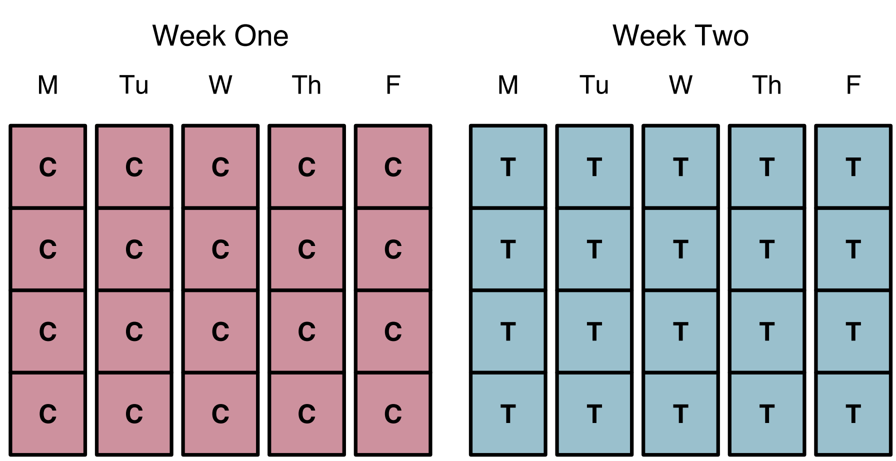
Example

- 20 male mice and 20 female mice.
- Half to be treated; the other half left untreated.
- Can only work with 4 mice per day.

Question:

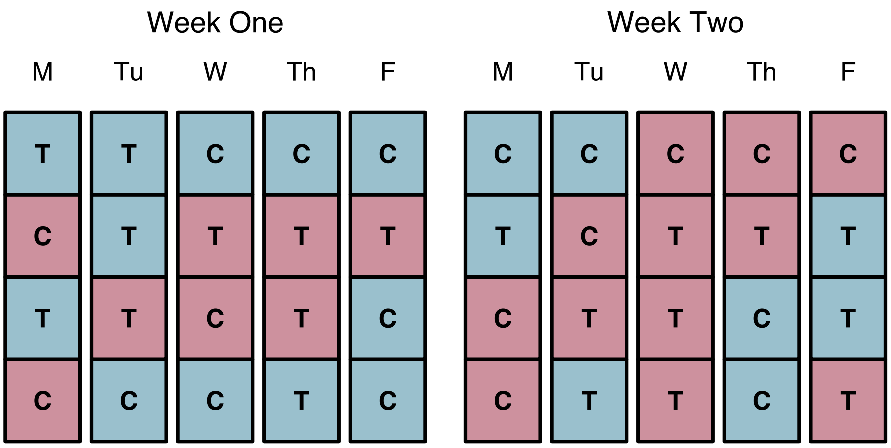
How to assign individuals to treatment groups and to days?

A really bad design



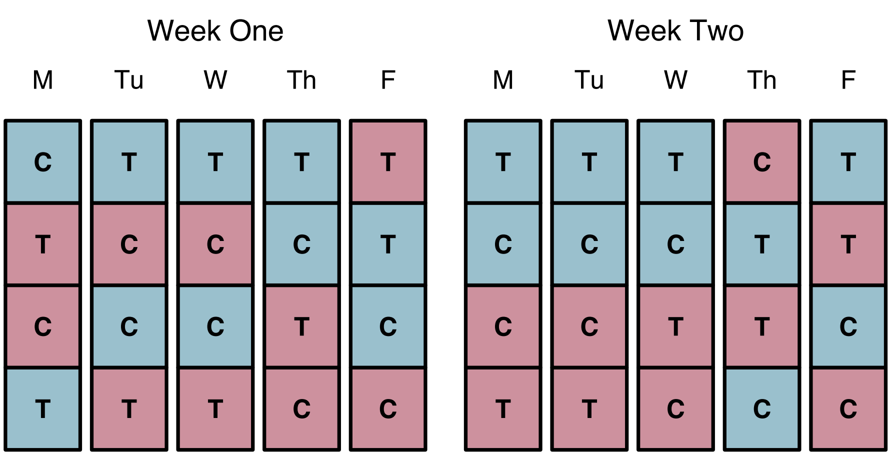
T = treated, C = control, pink = female, blue = male

A randomized design



T = treated, C = control, pink = female, blue = male

A stratified design



T = treated, C = control, pink = female, blue = male

Randomization and stratification

- If you can (and want to), fix a variable.

  E.g., use only 8 week old male mice from a single strain.

- If you don't fix a variable, stratify it.

  E.g., use 8 week and 12 week old male mice, and stratify with respect to age.

- If you can neither fix nor stratify a variable, randomize it.

Factorial experiments

Suppose we are interested in the effect of both salt water and a high-fat diet on blood pressure.

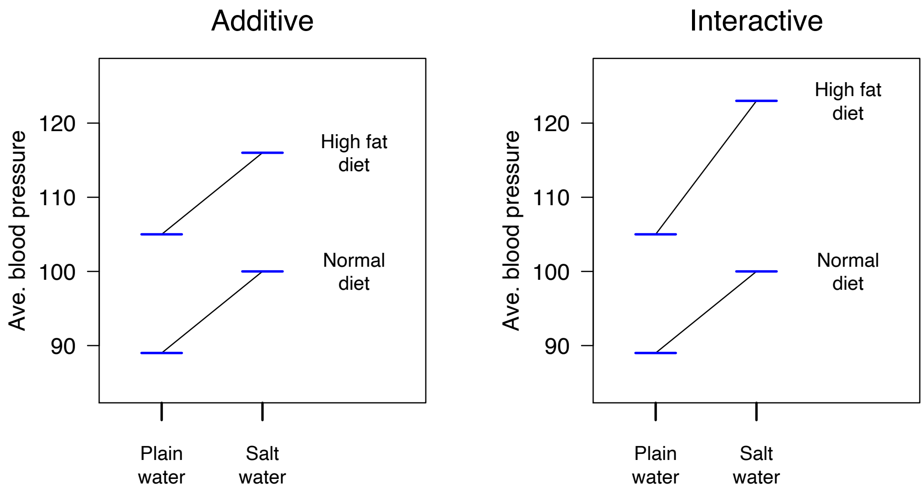
Ideally: look at all 4 treatments in one experiment.

$$\begin{matrix}\text{Plain water} \\ \text{Salt water}\end{matrix} \times \begin{matrix}\text{Normal diet} \\ \text{High-fat diet}\end{matrix}$$

Why?

- We can learn more.
- It is more efficient than doing all single-factor experiments.

Interactions



Other points

- Blinding

  Measurements made by people can be influenced by unconscious biases. Ideally, dissections and measurements should be made without knowledge of the treatment applied.

- Internal controls

  It can be useful to use the subjects themselves as their own controls (e.g., consider the response after vs. before treatment). Why? Increased precision.

- Representativeness

  Are the subjects/tissues you are studying really representative of the population you want to study? Ideally, your study material is a (stratified) random sample from the population of interest.

- Power

  Experimental design considerations (balanced designs!) and sample size calculations are crucial, in particular w/ regards to your predictor of interest.