Hypothesis generation and experimental design

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Today's talk

- Hypothesis generation
- Hypothesis testing
- Elements of good experimental design
- Tips on experimental design
- What comes after the experiment?

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Some different types of scientific work

- Measuring things (e.g. rainfall)
- Making things (e.g. expressing proteins in bacteria)
- Finding out how the universe operates (e.g. testing hypotheses about biology, physiology, disease)

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Assumptions about you

- That you are interested in hypothesis testing, leading to a need to design good experiments
- That you are doing “wet lab” type of work (i.e. not psychology, epidemiology)

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Experiment*: action or operation undertaken in order to discover something unknown, test hypothesis, etc.*

Science is nothing but trained and organised common sense, differing from the latter only as a veteran may differ from a raw recruit…..

(Thomas Huxley)

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Notes

Stages in designing and executing a set of experiments

**1. Formulate a hypothesis.*

**2. Design and execute an experiment or series of experiments to test (disprove) the hypothesis.*

3. Analyse and interpret the experimental results.

4. Reject hypothesis, or refine and re-test, or provisionally accept.

5. Report the conclusions.

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Formulating the hypothesis

Hypothesis: *supposition made as a basis for reasoning, or as a starting point for investigation.*

The great tragedy of Science - the slaying of a beautiful hypothesis by an ugly fact.

(Thomas Huxley)

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gather information

literature / own data / discussion / conference / supervisor

↓

organise information into tentative hypothesis

the first clever bit…..

↓

check whether hypothesis is original

literature search / discussion

↓

formalise the hypothesis

must be unambiguous - write it down!

↓

design experiment(s) to test hypothesis

another clever bit…..

Stages in designing an experiment

1. Have a hypothesis to test. Where possible we try to test hypotheses by *intervention*, rather than by *correlation*.

2. Decide what *treatments/interventions* to apply and what *outcomes* to measure.

3. Randomly assign "subjects" to experimental groups.

4. Decide on intervention / treatment schedule: e.g. order, frequency, dose.

5. Decide on schedule of observations / measurements.

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Elements of good experimental design

1. Discrimination: should test only one hypothesis.

2. Replication: enough subjects to allow for statistical tests of significance.

3. Controls: negative and positive.

4. Free of observer bias.

5. Measurements – accurate and precise.

6. Efficiency and practicability.

More detail coming…

Notes

1. Discrimination: should test only one hypothesis.

Example:

Observation: *substance X does not dissolve in water at room temperature.*

Hypothesis: *substance X will dissolve under alkaline conditions.*

Experiment: *place substance X in NaOH pH 9.0, heat to 50^{o} C for 1 hour.*

Outcome: *X dissolves.*

Has the hypothesis been properly tested?

2. Replication: enough subjects to allow for statistical tests of significance.

This is the realm of statistics!

At the outset you can calculate how many subjects you need in each experimental group to achieve a certain level of statistical significance when comparing data sets.

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3a. Controls: negative controls always, positive controls often.

Example A:

An animal is injected with a test drug dissolved in saline twice per day.

The negative control should be……..?

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3b. Controls: negative controls always, positive controls often.

Example B:

A hormone is added to cell line X.
The hypothesis is that it will increase the rate of cell division.
It does not do so.

Has the hypothesis been disproved?

The positive control(s) should have been……..?

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4. Free of observer bias.

Random assignment of subjects to experimental groups.

"Blinded" experiments, particularly when the measurement is subjective.

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5. Measurements: accurate and precise.

Accuracy: *does the method give you an unbiased value on average?*

Precision: *does the method give you a reproducible result on repetition?*

Common mistake: *if a balance reads to the nearest gram, don't express data means and errors to the nearest 0.1 gram.*

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6. Efficiency and practicability.

Ethics

Time

Cost

Expertise

Availability of reagents

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Tips on experimental design

- Try to test the hypothesis more than one way (especially when using so-called "specific" inhibitors)
- Consider possible sources of experimental error
- Try to obtain "hard" data, i.e. numerical read-outs, and avoid subjectivity
- Build in a "dry run" for procedures you haven't done before – at the very least, don't perform a very large, complex experiment first up
- Write up a <u>thorough</u> account of your reasoning, calculations, etc
- Write a simple protocol (e.g. a flow chart) to which you can refer "on the day"

What next (briefly)

- Apply appropriate statistical tests (and don't confuse "replicates" with "subjects")
- Plot your data: line charts, histograms; raw data, "% control"
- Is the reproducibility satisfactory?
- The problem of "outliers" – data cannot be rejected because we don't like what they say (use the ±2SD method)
- Repeat the experiment, if feasible
- How does the hypothesis look now?
- Discuss your data with others
- Report on your data and conclusions

Notes

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