

# Contrasts and multiple testing

## Session 4

MATH 80667A: Experimental Design and Statistical Methods  
HEC Montréal

# Outline

**Contrasts**

**Multiple testing**

# Planned comparisons

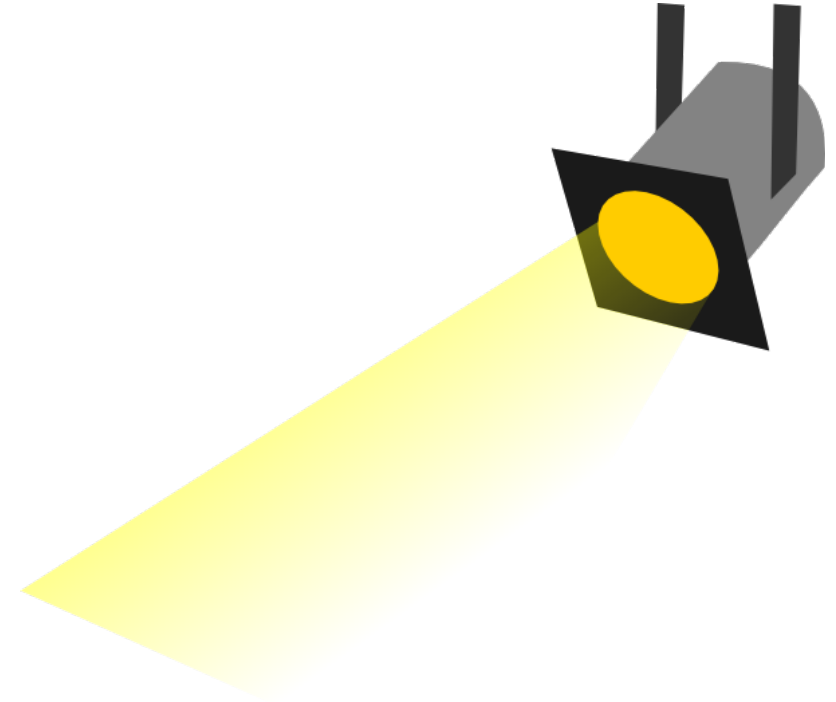
- Oftentimes, we are not interested in the global null hypothesis.
- Rather, we formulate planned comparisons *at registration time* for effects of interest

**What is the scientific question of interest?**

# Global null vs contrasts



**Global test**



**Contrasts**

# Linear contrasts

With  $K$  groups, null hypothesis of the form

$$\mathcal{H}_0 : C = c_1\mu_1 + \cdots + c_K\mu_K = a$$

weighted sum of subpopulation means

**Linear combination of  
weighted group averages**

# Examples of linear contrasts

Global mean larger than  $a$ ?

$$\mathcal{H}_0 : \frac{n_1}{n} \mu_1 + \cdots + \frac{n_K}{n} \mu_K \leq a$$

Pairwise comparison

$$\mathcal{H}_0 : \mu_i = \mu_j, \quad i \neq j$$

# Characterization of linear contrasts

- Weights  $c_1, \dots, c_K$  are specified by the **user**.
- Mean response in each experimental group is estimated as sample average of observations in that group,  $\hat{\mu}_1, \dots, \hat{\mu}_K$ .
- Assuming equal variance, the contrast statistic behaves in large samples like a Student- $t$  distribution with  $n - K$  degrees of freedom.

# Sum-to-zero constraint

If  $c_1 + \dots + c_K = 0$ , the contrast encodes

**differences between treatments**

rather than information about the overall mean.



# Arithmetic example

## Setup

group 1

(control)

group 2

(control)

group 3

(praise, reprove, ignore)

## Hypotheses of interest

- $\mathcal{H}_{01} : \mu_{\text{praise}} = \mu_{\text{reproved}}$  (attention)
- $\mathcal{H}_{02} : \frac{1}{2} (\mu_{\text{control}_1} + \mu_{\text{control}_2}) = \mu_{\text{praised}}$  (encouragement)

# Contrasts

With placeholders for each group, write  $\mathcal{H}_{01} : \mu_{\text{praised}} = \mu_{\text{reproved}}$  as

$$0 \cdot \mu_{\text{control}_1} + 0 \cdot \mu_{\text{control}_2} + 1 \cdot \mu_{\text{praised}} - 1 \cdot \mu_{\text{reproved}} + 0 \cdot \mu_{\text{ignored}}$$

The sum of the coefficient vector,  $\mathbf{c} = (0, 0, 1, -1, 0)$ , is zero.

Similarly, for  $\mathcal{H}_{02} : \frac{1}{2}(\mu_{\text{control}_1} + \mu_{\text{control}_2}) = \mu_{\text{praise}}$

$$\frac{1}{2} \cdot \mu_{\text{control}_1} + \frac{1}{2} \cdot \mu_{\text{control}_2} - 1 \cdot \mu_{\text{praised}} + 0 \cdot \mu_{\text{reproved}} + 0 \cdot \mu_{\text{ignored}}$$

The contrast vector is  $\mathbf{c} = (\frac{1}{2}, \frac{1}{2}, -1, 0, 0)$ ; entries again sum to zero.

Equivalent formulation is obtained by picking  $\mathbf{c} = (1, 1, -2, 0, 0)$

# Contrasts in R with emmeans

```
library(emmeans)
linmod <- lm(score ~ group, data = arithmetic)
linmod_emm <- emmeans(linmod, specs = 'group')
contrast_specif <- list(
  controlvspraised = c(0.5, 0.5, -1, 0, 0),
  praisedvsreproved = c(0, 0, 1, -1, 0)
)
contrasts_res <-
  contrast(object = linmod_emm,
           method = contrast_specif)
# Obtain confidence intervals instead of p-values
confint(contrasts_res)
```

# Output

<b>contrast</b>	<b>null.value</b>	<b>estimate</b>	<b>std.error</b>	<b>df</b>	<b>statistic</b>	<b>p.value</b>
control vs praised	0	-8.44	1.40	40	-6.01	<1e-04
praised vs reprove	0	4.00	1.62	40	2.47	0.018

## Confidence intervals

<b>contrast</b>	<b>lower</b>	<b>upper</b>
control vs praised	-11.28	-5.61
praised vs reprove	0.72	7.28

# One-sided tests

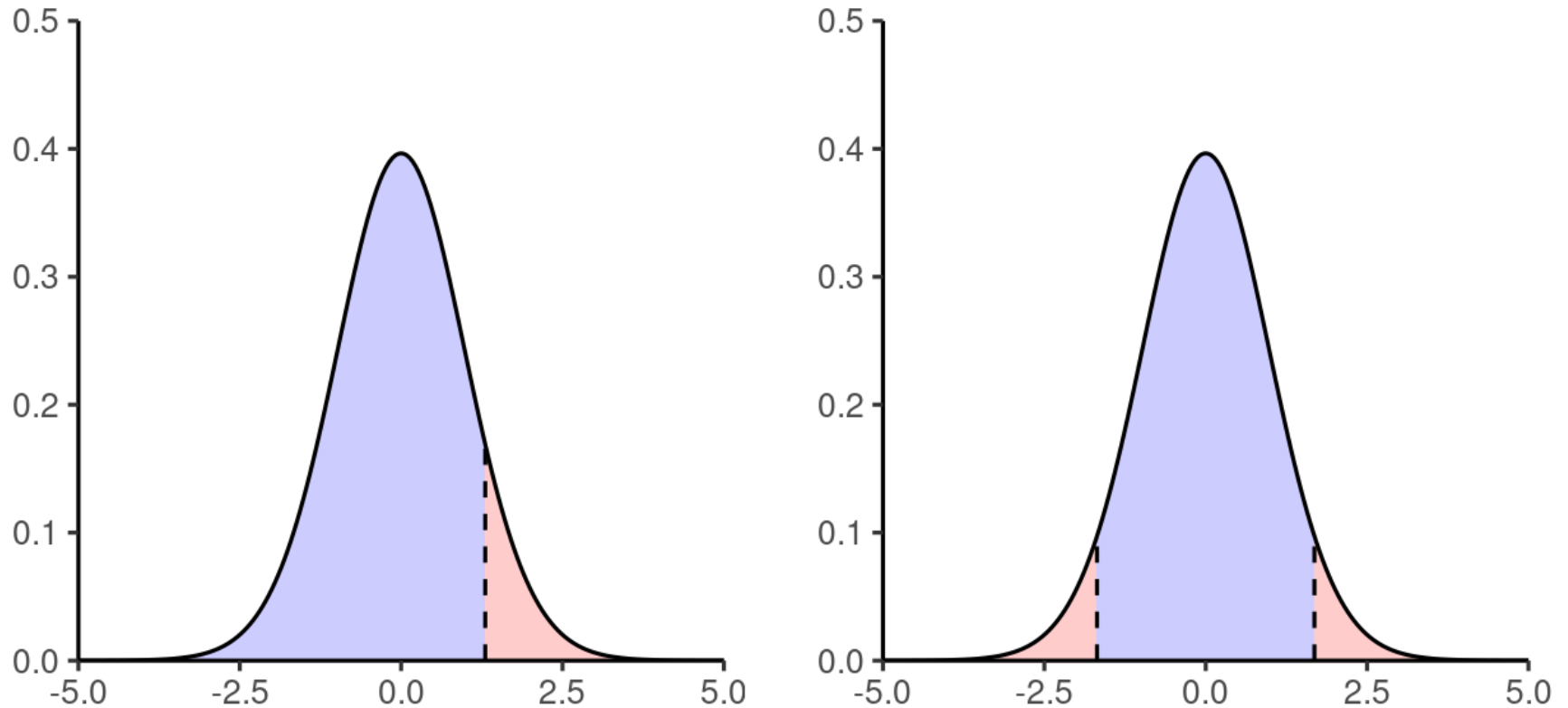
Suppose we postulate that the contrast statistic is **bigger** than some value  $a$ .

- The alternative is  $\mathcal{H}_a : C > a$  (what we are trying to prove)!
- The null hypothesis is therefore  $\mathcal{H}_0 : C \leq a$  (Devil's advocate)

It suffices to consider the endpoint  $C = a$  (why?)

- If we reject  $C = a$  in favour of  $C > a$ , all other values of the null hypothesis are even less compatible with the data.

# Comparing rejection regions



Rejection regions for a one-sided test (left) and a two-sided test (right).

# When to use one-sided tests?

In principle, one-sided tests are more powerful (larger rejection region on one sided).

- However, important to **pre-register** hypothesis
  - can't look at the data before formulating the hypothesis (as always)!
- More logical for follow-up studies and replications.

If you postulate  $\mathcal{H}_a : C > a$  and the data show the opposite with  $\hat{C} \leq a$ , then the  $p$ -value for the one-sided test is 1!

# Multiple testing



# Post-hoc tests

Suppose you decide to look at all pairwise differences

**Comparing all pairwise differences:  $m = \binom{K}{2}$  tests**

- $m = 3$  tests if  $K = 3$  groups,
- $m = 10$  tests if  $K = 5$  groups,
- $m = 45$  tests if  $K = 10$  groups...

# There is a catch...

Read the small prints:

If you do a **single** hypothesis test and your testing procedure is well calibrated (*meaning the model assumptions are met*), there is a probability  $\alpha$  of making a type I error if the null hypothesis is true.

# How many tests?

Dr. Yoav Benjamini looked at the number of tests performed in the Psychology replication project

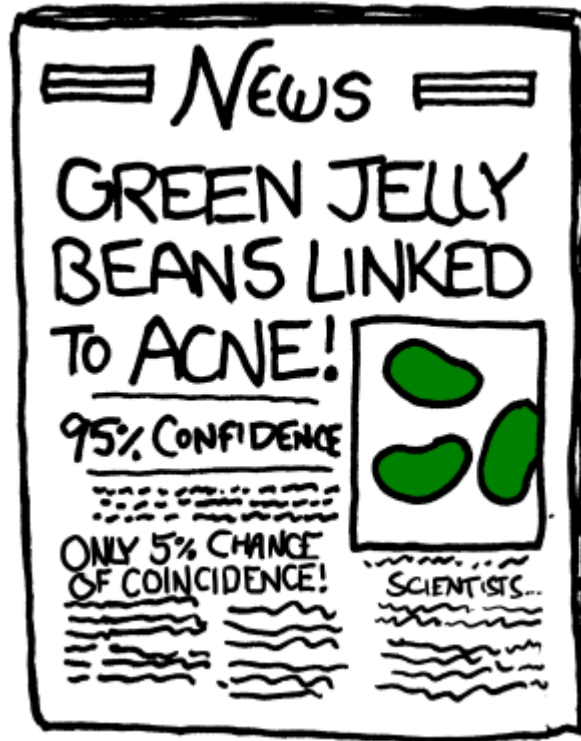
Open Science Collaboration. (2015). Estimating the reproducibility of psychological science. *Science*, 349(6251), aac4716.

The number of tests performed ranged from 4 to 700, with an average of 72.

Most studies did not account for selection.

# Scientist, investigate!

- Consider the Cartoon *Significant* by Randall Munroe (<https://xkcd.com/882/>)



It highlights two problems: lack of accounting for multiple testing and selective reporting.

# Probability of type I error

If we do  $m$  **independent** comparisons, each one at the level  $\alpha$ , the probability of making at least one type I error, say  $\alpha^*$ , is

$$\alpha^* = 1 - \text{probability of making no type I error} = 1 - (1 - \alpha)^m.$$

With  $\alpha = 0.05$

- $m = 4$  tests,  $\alpha^* \approx 0.185$ .
- $m = 72$  tests,  $\alpha^* \approx 0.975$ .

Tests need not be independent... but one can show  $\alpha^* \leq m\alpha$ .

# Statistical significance at the 5% level

Why  $\alpha = 5\%$ ? Essentially **arbitrary**...

If one in twenty does not seem high enough odds, we may, if we prefer it, draw the line at one in fifty or one in a hundred. Personally, the writer prefers to set a low standard of significance at the 5 per cent point, and ignore entirely all results which fails to reach this level.

Fisher, R.A. (1926). The arrangement of field experiments. *Journal of the Ministry of Agriculture of Great Britain*, 33:503-513.

# Family of hypothesis

Consider  $m$  tests with the corresponding null hypotheses  $\mathcal{H}_{01}, \dots, \mathcal{H}_{0m}$ .

- The family may depend on the context, but including any hypothesis that is scientifically relevant and could be reported.

**Should be chosen a priori and pre-registered**

**Keep it small:** the number of planned comparisons for a one-way ANOVA should be less than the number of groups  $K$ .

# Notation

Define indicators

$$R_i = \begin{cases} 1 & \text{if we reject } \mathcal{H}_{0i} \\ 0 & \text{if we fail to reject } \mathcal{H}_{0i} \end{cases}$$

$$V_i = \begin{cases} 1 & \text{type I error for } \mathcal{H}_{0i} \quad (R_i = 1 \text{ and } \mathcal{H}_{0i} \text{ is true}) \\ 0 & \text{otherwise} \end{cases}$$

with

- $R = R_1 + \cdots + R_m$  the total number of rejections ( $0 \leq R \leq m$ ).
- $V = V_1 + \cdots + V_m$  the number of null hypothesis rejected by mistake.



# Familywise error rate

**Definition:** the familywise error rate is the probability of making at least one type I error per family

$$\text{FWER} = \Pr(V \geq 1)$$

If we use a procedure that controls for the family-wise error rate, we talk about **simultaneous inference** (or simultaneous coverage for confidence intervals).

# Bonferroni's procedure

Consider a family of  $m$  hypothesis tests and perform each test at level  $\alpha/m$ .

- reject  $i$ th null  $\mathcal{H}_{i0}$  if the associated  $p$ -value  $p_i \leq \alpha/m$ .
- build confidence intervals similarly with  $1 - \alpha/m$  quantiles.

If the (raw)  $p$ -values are reported, reject  $\mathcal{H}_{0i}$  if  $m \times p_i \leq \alpha$  (i.e., multiply reported  $p$ -values by  $m$ )

# Holm's sequential method

Order the  $p$ -values of the family of  $m$  tests from smallest to largest

$$p_{(1)} \leq \dots \leq p_{(m)}$$

associated to null hypothesis  $\mathcal{H}_{0(1)}, \dots, \mathcal{H}_{0(m)}$ .

**Idea** use a different level for each test, more stringent for smaller  $p$ -values.

Coupling Holm's method with Bonferroni's procedure: compare  $p_{(1)}$  to  $\alpha_{(1)} = \alpha/m$ ,  $p_{(2)}$  to  $\alpha_{(2)} = \alpha/(m - 1)$ , etc.

**Holm-Bonferroni procedure is always more powerful than Bonferroni**

# Sequential Holm-Bonferroni procedure

1. order  $p$ -values from smallest to largest.
2. start with the smallest  $p$ -value.
3. check significance one test at a time.
4. stop when the first non-significant  $p$ -value is found or no more test.

# Conclusion for Holm-Bonferroni

Reject smallest  $p$ -values until you find one that fails, reject rest

If  $p_{(j)} \geq \alpha_{(j)}$  but  $p_{(i)} < \alpha_{(i)}$  for  $i = 1, \dots, j - 1$  (all smaller  $p$ -values)

- reject  $\mathcal{H}_{0(1)}, \dots, \mathcal{H}_{0(j-1)}$
- fail to reject  $\mathcal{H}_{0(j)}, \dots, \mathcal{H}_{0(m)}$

All  $p$ -values are lower than their respective cutoff:

If  $p_{(i)} \leq \alpha_{(i)}$  for all test  $i = 1, \dots, m$

- reject  $\mathcal{H}_{0(1)}, \dots, \mathcal{H}_{0(m)}$

# Numerical example

Consider  $m = 3$  tests with raw  $p$ -values 0.01, 0.04, 0.02.

$i$	$p_{(i)}$	Bonferroni	Holm-Bonferroni
1	0.01	$3 \times 0.01 = 0.03$	$3 \times 0.01 = 0.03$
2	0.02	$3 \times 0.02 = 0.06$	$2 \times 0.02 = 0.04$
3	0.04	$3 \times 0.04 = 0.12$	$1 \times 0.04 = 0.04$

Reminder of Holm-Bonferroni: multiply by  $(m - i + 1)$  the  $i$ th smallest  $p$ -value  $p_{(i)}$ , compare the product to  $\alpha$ .

# Why choose Bonferroni's procedure?

- $m$  must be prespecified
- simple and generally applicable (any design)
- but dominated by sequential procedures (Holm-Bonferroni uniformly more powerful)
- low power when the number of test  $m$  is large
- also controls for the expected number of false positive,  $E(V)$ , a more stringent criterion called **per-family error rate** (PFER)

**Careful:** adjust for the real number of comparisons made (often reporter just correct only the 'significant tests', which is wrong).

# Confidence intervals for linear contrasts

Given a linear contrast of the form

$$C = c_1\mu_1 + \cdots + c_K\mu_K$$

with  $c_1 + \cdots + c_K = 0$ , we build confidence intervals as usual

$$\hat{C} \pm \text{critical value} \times \widehat{\text{se}}(\hat{C})$$

Different methods provide control for FWER by modifying the **critical value**.

All methods valid with equal group variances and independent observations.



# FWER control in ANOVA

- **Tukey**'s honestly significant difference (HSD) method: to compare (all) pairwise differences between subgroups, based on the largest possible pairwise mean differences, with extensions for unbalanced samples.
- **Scheffé**'s method: applies to any contrast (properties depends on sample size  $n$  and number of groups  $K$ , not the number of test). Better than Bonferroni if  $m$  is large. Can be used for any design, but not powerful.
- **Dunnett**'s method: only for all pairwise contrasts relative to a specific baseline (control).

Described in Dean, Voss and Draguljić (2017), Section 4.4 in more details.

# Tukey's honest significant difference

**Control for all pairwise comparisons**

Idea: controlling for the range

$$\max\{\mu_1, \dots, \mu_K\} - \min\{\mu_1, \dots, \mu_K\}$$

automatically controls FWER for other pairwise differences.

**Critical values based on "Studentized range" distribution**

Assumptions: equal variance, equal number of observations in each experimental condition.

# Scheffé's criterion

**Control for all possible linear contrasts**

Critical value is  $\sqrt{(K - 1)F}$ ,  
where  $F$  is the  $(1 - \alpha)$  quantile  
of the  $F(K - 1, n - K)$  distribution.

**Allows for data snooping  
(post-hoc hypothesis)**

**But not powerful...**

# Adjustment for one-way ANOVA

## Take home message:

- same as usual, only with different **critical values**
- larger cutoffs for  $p$ -values when procedure accounts for more tests

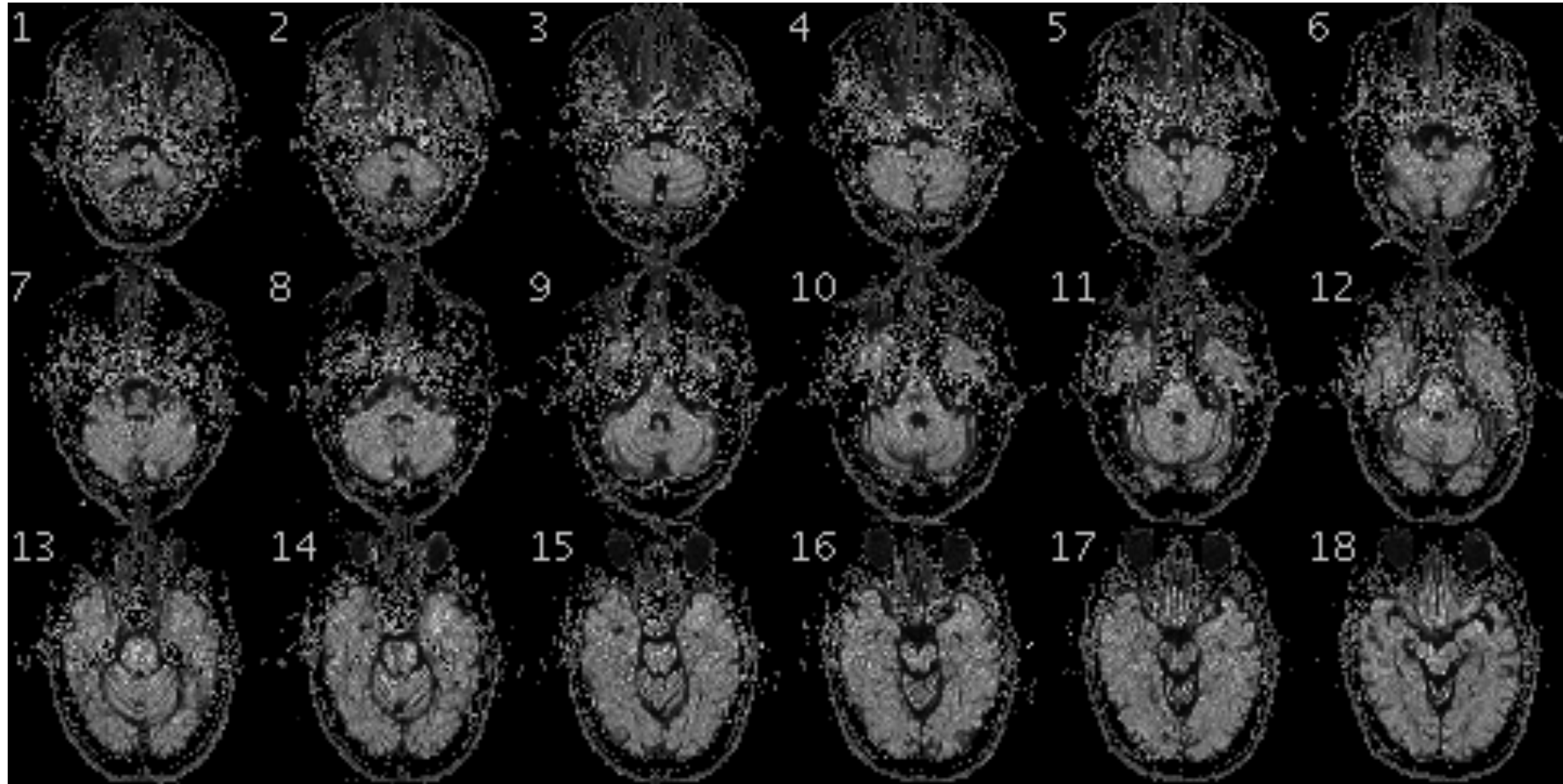
Everything is obtained using software.

# Numerical example

With  $K = 5$  groups and  $n = 9$  individuals per group (arithmetic example), critical value for two-sided test of zero difference with standardized  $t$ -test statistic and  $\alpha = 5\%$  are

- Scheffé's (all contrasts): 3.229
- Tukey's (all pairwise differences): 2.856
- Dunnett's (difference to baseline): 2.543
- unadjusted Student's  $t$ -distribution: 2.021

# Sometimes, there are too many tests...



# Scaling back expectations...

A simultaneous procedure that controls family-wise error rate (FWER) ensure any selected test has type I error  $\alpha$ .

With thousands of tests, this is too stringent a criterion.

The false discovery rate (FDR) provides a guarantee for the proportion **among selected** discoveries (tests for which we reject the null hypothesis).

Why use it? the false discovery rate is scalable:

- 2 type I errors out of 4 tests is unacceptable.
- 2 type I errors out of 100 tests is probably okay.

# False discovery rate

Suppose that  $m_0$  out of  $m$  null hypothesis are true

The **false discovery rate** is the proportion of false discovery among rejected nulls,

$$\text{FDR} = \begin{cases} \frac{V}{R} & R > 0 \text{ (if one or more rejection),} \\ 0 & R = 0 \text{ (if no rejection).} \end{cases}$$



# Controlling false discovery rate

The Benjamini-Hochberg (1995) procedure for controlling false discovery rate is:

1. Order the  $p$ -values from the  $m$  tests from smallest to largest:

$$p_{(1)} \leq \cdots \leq p_{(m)}$$

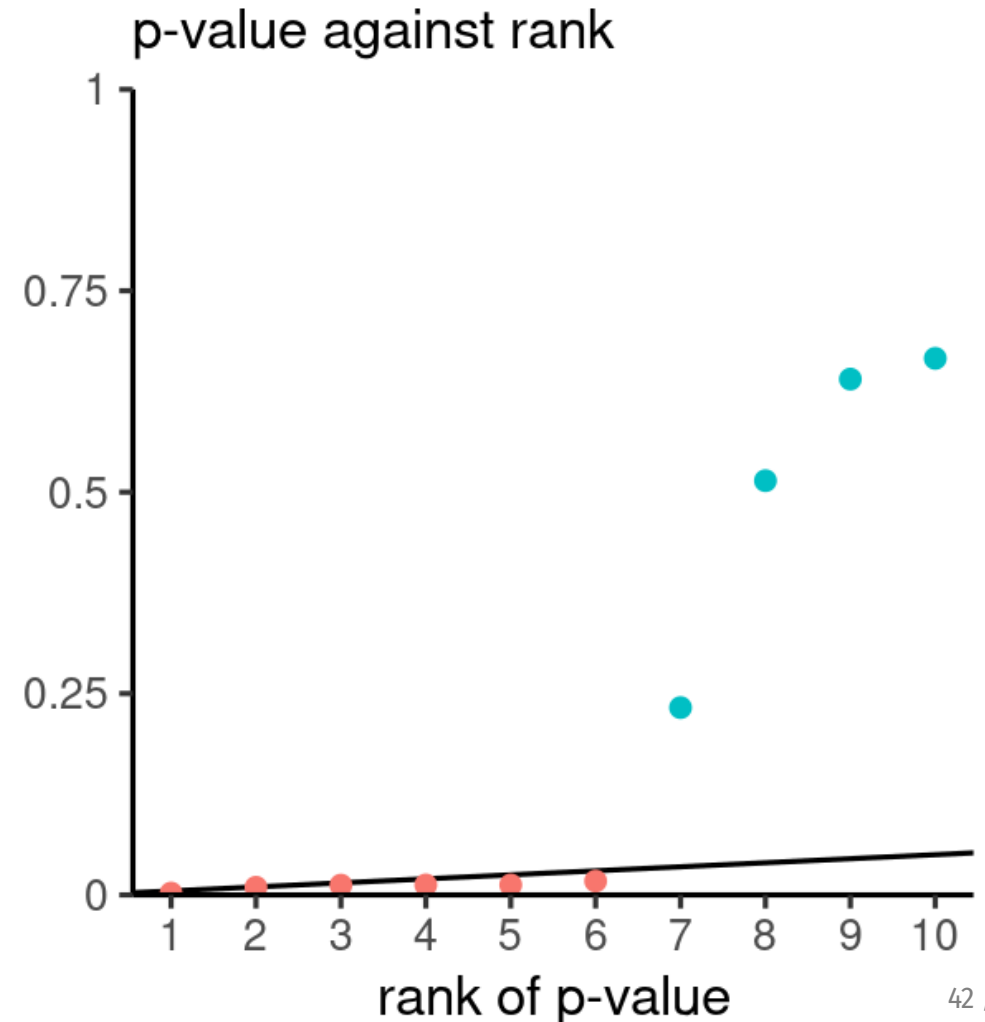
2. For level  $\alpha$  (e.g.,  $\alpha = 0.05$ ), set

$$k = \max \left\{ i : p_{(i)} \leq \frac{i}{m} \alpha \right\}$$

3. Reject  $\mathcal{H}_{0(1)}, \dots, \mathcal{H}_{0(k)}$ .

# Benjamini-Hochberg in a picture

1. Plot  $p$ -values ( $y$ -axis) against their rank ( $x$ -axis)
  - (the smallest  $p$ -value has rank 1, the largest has rank  $m$ ).
2. Draw the line  $y = \alpha/mx$ 
  - (zero intercept, slope  $\alpha/m$ )
3. Reject all null hypotheses associated to  $p$ -values located before the first time a point falls above the line.



# Recap 1

- The test of equality of variance of the one-way ANOVA is seldom of interest (too general or vague)
- Rather, we care about specific comparisons (often linear contrasts)
- Must specify ahead of time which comparisons are of interest
  - otherwise it's easy to find something significant!
  - and multiplicity correction will be unfavorable.

# Recap 2

- Researchers often carry lots of hypothesis testing tests
  - the more you look, the more you find!
  - One of the many reasons for the replication crisis!
- Thus want to control probability of making a type I error (condemn innocent, incorrect finding) among all  $m$  tests performed
  - aka family-wise error rate (FWER)
  - Downside of multiplicity correction/adjustment is loss of power
  - upside is (more robust findings).

# Recap 3

ANOVA specific solutions (assuming equal variance, balanced large samples...)

- Tukey's HSD (all pairwise differences),
- Dunnett's method (only differences relative to a reference category)
- Scheffé's method (all potential linear contrasts)

Outside of ANOVA, some more general recipes:

- FWER: Bonferroni (suboptimal), Bonferroni-Holm (more powerful)
- FDR: Benjamini-Hochberg

Pick the one that controls FWER, but penalizes less!