

STA305/1004-Class 17

Nov. 21, 2019

Today's Class

ANOVA

- ▶ Multiple comparisons
- ▶ Sample size for ANOVA

Multiple Comparisons

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Multiple Comparisons

all pairwise comparisons.

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$$\underbrace{\hspace{1.5cm}}_A = 1 - P(A^c)$$

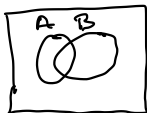
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$$A_k = \{ \text{reject } H_{0_k} \}$$

$$P(\text{reject at least one } H_{0_k}) = P\left(\bigcup_{k=1}^3 A_k\right)$$

$$(A \cup B)^c = A^c \cap B^c$$



$$\begin{aligned} &= 1 - P\left(\left(\bigcup_{k=1}^3 A_k\right)^c\right) \\ &= 1 - P\left(\bigcap_{k=1}^3 A_k^c\right) \end{aligned}$$

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 $1 - P(\text{do not reject } H_{0_1} \text{ and do not reject } H_{0_2} \text{ and do not reject } H_{0_3})$

$$1 - P(A_1^c) P(A_2^c) P(A_3^c) \quad A_{0k} = \{\text{reject } H_{0k}\}$$

assuming that A_1, A_2, A_3
are independent.

$$= 1 - (1 - \alpha)(1 - \alpha)(1 - \alpha) = 1 - (1 - \alpha)^3$$

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Multiple Comparisons

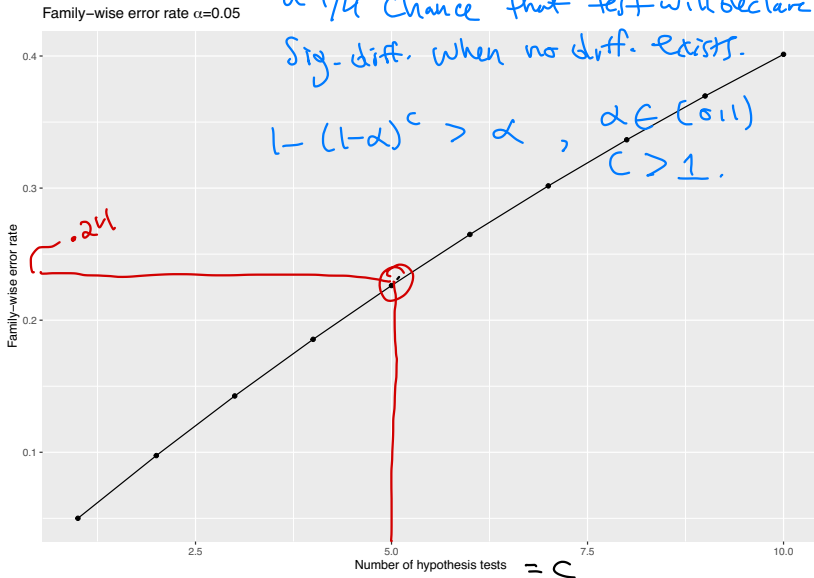
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- ▶ If $\alpha = 0.05$ then the probability that at least one H_0 will be falsely rejected is $1 - (1 - .05)^3 = 0.14$, which is almost three times the type I error rate.

Multiple Comparisons

For example if $C=5$ then there is almost a $1/4$ chance that test will declare a sig. diff. when no diff. exists.

$$1 - (1 - \alpha)^C > \alpha, \quad \alpha \in (0, 1), \quad C > 1.$$

$$1 - (1 - \alpha)^C$$



Multiple Comparisons

$$H_0 : \mu_1 = \mu_2 = \cdots = \mu_k \text{ vs. } H_1 : \mu_i \neq \mu_j.$$

If c independent hypotheses are conducted then the probability

$$P(\text{reject at least one } H_{0_k}) = 1 - (1 - \alpha)^c$$

is called the **family-wise error rate**.

The **pairwise error rate** is $P(\text{reject } H_{0_k}) = \alpha$ for any c .

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The Multiple Comparisons Problem

- ▶ The multiple comparison problem is that multiple hypotheses are tested level α which increases the probability that at least one of the hypotheses will be falsely rejected (family-wise error rate).
- ▶ If treatment means are significantly different from the ANOVA F test then researchers usually want to explore which means are different.
- ▶ Is it appropriate to test for differences looking at all pairwise comparisons?
- ▶ Testing all possible pairs increases the type I error rate.
- ▶ This means that there is a higher probability, beyond the pre-stated type I error rate (e.g. 0.05), that that a significant difference is detected when the truth is that no difference exists.

Example



Neural correlates of interspecies perspective taking in the post-mortem Atlantic Salmon: An argument for multiple comparisons correction

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INTRODUCTION

With the extreme dimensionality of functional neuroimaging data comes extreme risk for false positives. Across the 130,000 voxels in a typical fMRI volume the probability of a false positive is almost certain. Correction for multiple comparisons should be completed with these datasets, but is often ignored by investigators. To illustrate the magnitude of the problem we carried out a real experiment that demonstrates the danger of not correcting for chance properly.

METHODS

Subject. One mature Atlantic Salmon (*Salmo salar*) participated in the fMRI study. The salmon was approximately 18 inches long, weighed 3.8 lbs, and was not alive at the time of scanning.

Task. The task administered to the salmon involved completing an open-ended mentalizing task. The salmon was shown a series of photographs depicting human individuals in social situations with a specified emotional valence. The salmon was asked to determine what emotion the individual in the photo must have been experiencing.

Design. Stimuli were presented in a block design with each photo presented for 10 seconds followed by 12 seconds of rest. A total of 15 photos were displayed. Total scan time was 5.5 minutes.

Processing. Image processing was completed using SPM1. Preprocessing steps for the functional imaging data included a 6-parameter rigid-body affine realignment of the fMRI timeseries, coregistration of the data to a T₁-weighted anatomical image, and 8 mm full-width at half-maximum (FWHM) Gaussian smoothing.

Analysis. Voxelwise statistics on the salmon data were calculated through an ordinary least-squares estimation of the general linear model (GLM). Predictions of the hemodynamic response were modeled by a boxcar function convolved with a canonical hemodynamic response. A temporal high pass filter of 128 seconds was included to account for low frequency drift. No autocorrelation correction was applied.

Voxel Selection. Two methods were used for the correction of multiple comparisons in the fMRI results. The first method controlled the overall false discovery rate (FDR) and was based on a method defined by Benjamini and Hochberg (1995). The second method controlled the overall familywise error rate (FWER) through the use of Gaussian random field theory. This was done using algorithms originally devised by Friston et al. (1994).

DISCUSSION

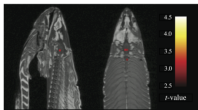
Can we conclude from this data that the salmon is engaging in the perspective-taking task? Certainly not. What we can determine is that random noise in the EPI timeseries may yield spurious results if multiple comparisons are not controlled for. Adaptive methods for controlling the FDR and FWER are excellent options and are widely available in all major fMRI analysis packages. We argue that relying on standard statistical thresholds ($p < 0.001$) and low minimum cluster sizes ($k > 8$) is an ineffective control for multiple comparisons. We further argue that the vast majority of fMRI studies should be utilizing multiple comparisons correction as standard practice in the computation of their statistics.

REFERENCES

Benjamini Y and Hochberg Y (1995). Controlling the false discovery rate: a practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society: Series A*, 57:289-300.

Friston KJ, Worsley KJ, Fradette J, Martinez JC, and Evans AC. (1994). Assessing the significance of focal activations using spatial extent. *Human Brain Mapping*, 1:214-228.

GLM RESULTS

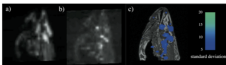


A t -contrast was used to test for regions with significant BOLD signal change during the photo condition compared to rest. The parameters for this comparison were $\alpha(131) > 3.15$, $\mu(\text{uncorrected}) < 0.001$, 3 voxel extent threshold.

Several active voxels were discovered in a cluster located within the salmon's brain cavity (Figure 1, see above). The size of this cluster was 81 mm³ with a cluster-level significance of $p = 0.001$. Due to the coarse resolution of the echo-planar image acquisition and the relatively small size of the salmon brain further discrimination between brain regions could not be completed. Out of a search volume of 8064 voxels a total of 16 voxels were significant.

Identical t -contrasts controlling the false discovery rate (FDR) and familywise error rate (FWER) were completed. These contrasts indicated no active voxels, even at relaxed statistical thresholds ($p = 0.25$).

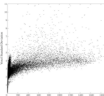
VOXEL-WISE VARIABILITY



To examine the spatial configuration of false positives we completed a voxelwise analysis of the fMRI timeseries. On a voxel-by-voxel basis we calculated the standard deviation of signal values across all 140 volumes.

We observed clustering of highly variable voxels into groups near areas of high voxel signal intensity. Figure 2a shows the mean EPI image for all 140 image volumes. Figure 2b shows the standard deviation values of each voxel. Figure 2c shows thresholded standard deviation values overlaid onto a high-resolution T₁-weighted image.

To investigate this effect in greater detail we conducted a Pearson correlation to examine the relationship between the signal in a voxel and its variability. There was a significant positive correlation between the mean voxel value and its variability over time ($r = 0.54$, $p < 0.001$). A scatterplot of mean voxel signal intensity against voxel standard deviation is presented to the right.



The Bonferroni Method

To test for the difference between the i th and j th treatments, it is common to use the two-sample t test. The two-sample t statistic is

$$t_{ij} = \frac{\bar{y}_{j\cdot} - \bar{y}_{i\cdot}}{\hat{\sigma} \sqrt{1/n_j + 1/n_i}},$$

where $\bar{y}_{j\cdot}$ is the average of the n_j observations for treatment j and $\hat{\sigma}$ is $\sqrt{MS_E}$ from the ANOVA table.

Treatments i and j are declared significantly different at level α if

$$|t_{ij}| > \underbrace{t_{N-k, \alpha/2}}_{\text{critical value}},$$

where $t_{N-k, \alpha/2}$ is the upper $\alpha/2$ percentile of a t_{N-k} .

The Bonferroni Method

The total number of pairs of treatment means that can be tested is

$$c = \binom{k}{2} = \frac{k(k-1)}{2}.$$

The Bonferroni method for testing $H_0 : \mu_i = \mu_j$ vs. $H_0 : \mu_i \neq \mu_j$ rejects H_0 at level α if

$$|t_{ij}| > t_{N-k, \alpha/2c},$$

where c denotes the number of pairs being tested.

Instead of using
 $\alpha/2$ use
 $\alpha/\alpha \cdot c$

The Bonferroni Method

In R the function `pairwise.t.test()` can be used to compute Bonferroni adjusted p-values.

This is illustrated below for the blood coagulation study.

```
pairwise.t.test(tab0401$y, tab0401$diets, p.adjust.method = "bonferroni")
```

```
##  
## Pairwise comparisons using t tests with pooled SD  
##  
## data:  tab0401$y and tab0401$diets  
##  
##      A      B      C  
## B 0.00934 -      -  
## C 0.00031 0.95266 -  
## D 1.00000 0.00934 0.00031  
##  
## P value adjustment method: bonferroni
```

Coagulation time

diets

Adjust
using
bonferroni

p-value for testing $H_0: \mu_A - \mu_B = 0$
but using
 $\alpha = 0.05/2.6$

$H_A: \mu_A - \mu_B \neq 0$

There are significant differences at the 5% level between diets A and B, A and C, B and D, and C and D using the Bonferroni method.

The Bonferroni Method

For comparison the unadjusted p-values are also calculated.

```
pairwise.t.test(tab0401$y, tab0401$diets, p.adjust.method = "none")
```

```
##  
## Pairwise comparisons using t tests with pooled SD  
##  
## data: tab0401$y and tab0401$diets  
##  
##      A      B      C  
## B 0.0016 -      -  
## C 5.2e-05 0.1588 -  
## D 1.0000 0.0016 5.2e-05  
##  
## P value adjustment method: none
```

no
adjustment
for MC.

The significant differences are the same using the unadjusted p-values but the p-values are larger than the p-values adjusted using the Bonferroni method.

The Bonferroni Method

A $100(1 - \alpha)\%$ simultaneous confidence interval for c pairs $\mu_i - \mu_j$ is

$$\bar{y}_{j\cdot} - \bar{y}_{i\cdot} \pm t_{N-k, \alpha/2c} \hat{\sigma} \sqrt{1/n_j + 1/n_i}.$$

After identifying which pairs are different, the confidence interval quantifies the range of plausible values for the differences.

The Bonferroni Method - coagulation study

The treatment means can be obtained from the table below.

	A	B	C	D
	60	65	71	62
	63	66	66	60
	59	67	68	61
	63	63	68	64
	62	64	67	63
	59	71	68	56
Treatment Average	61	66	68	61
Grand Average	64	64	64	64
Difference	-3	2	4	-3

The Bonferroni Method - coagulation study

$\hat{\sigma} = \sqrt{MS_E}$ can be obtained from the ANOVA table.

```
anova(lm(y~diets,data=tab0401))
```

```
## Analysis of Variance Table
```

```
##
```

```
## Response: y
```

```
##          Df Sum Sq Mean Sq F value    Pr(>F)
## diets      3    228    76.0    13.571 4.658e-05 ***
## Residuals 20    112     5.6
```

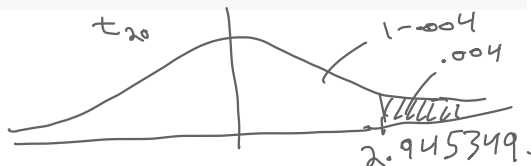
```
## ---
```

```
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

The upper $.05/(2 \cdot 6) = 0.004$ percentile of the t_{24-4} can be obtained with the t quantile function in R qt().

```
qt(p = 1-0.004,df = 20)
```

```
## [1] 2.945349
```



The Bonferroni Method - coagulation study

Plugging in these values to the confidence interval formula we can obtain a Bonferroni adjusted 95% confidence interval for $\mu_B - \mu_A$:

$$66 - 61 \pm 2.95\sqrt{5.6}\sqrt{1/6 + 1/6}$$

The lower and upper limits can be calculated in R.

```
66-61 - qt(p = 1-0.004,df = 20)*sqrt(5.6)*sqrt(1/6+1/6) # lower limit
```

```
## [1] 0.9758869
```

```
66-61 + qt(p = 1-0.004,df = 20)*sqrt(5.6)*sqrt(1/6+1/6) # upper limit
```

```
## [1] 9.024113
```

The 95% confidence interval for $\mu_B - \mu_A$ is (0.98, 9.02).

↑ adjusted for
MC using Bonferroni

The Tukey Method

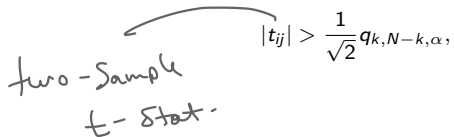
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$$|t_{ij}| > \frac{1}{\sqrt{2}} q_{k, N-k, \alpha},$$

two-sample
t-stat.

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$$|t_{ij}| > \frac{1}{\sqrt{2}} q_{k, N-k, \alpha},$$

- ▶ t_{ij} is the observed value of the two-sample t-statistic

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- ▶ t_{ij} is the observed value of the two-sample t-statistic
- ▶ $q_{k, N-k, \alpha}$ is the upper α percentile of the Studentized range distribution with parameters k and $N - k$ degrees of freedom.

another
olst. useful
for m.c.

↑
treatments

Number of obs. - # treatments

The Tukey Method

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▶

$$|t_{ij}| > \frac{1}{\sqrt{2}} q_{k, N-k, \alpha},$$

- ▶ t_{ij} is the observed value of the two-sample t-statistic
- ▶ $q_{k, N-k, \alpha}$ is the upper α percentile of the Studentized range distribution with parameters k and $N - k$ degrees of freedom.
- ▶ The CDF and inverse CDF of the Studentized Range Distribution is available in R via the functions `ptukey()` and `qtukey()` respectively.

The Tukey Method

family-wise error rate
is α .

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$$\underbrace{\bar{y}_j - \bar{y}_i}_{\text{est}} \pm \underbrace{\frac{1}{\sqrt{2}} q_{k, N-k, \alpha}}_{\substack{\text{error} \\ \downarrow \\ \text{crit. value}}} \underbrace{\hat{\sigma} \sqrt{\frac{1}{n_j} + \frac{1}{n_i}}}_{\substack{\text{s.e.} \\ \hat{\sigma} \sqrt{\frac{1}{n_j} + \frac{1}{n_i}}}}$$

The Tukey Method

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$$\bar{y}_{j\cdot} - \bar{y}_{i\cdot} \pm \frac{1}{\sqrt{2}} q_{k, N-k, \alpha} \hat{\sigma} \sqrt{1/n_j + 1/n_i}.$$

- ▶ The Bonferroni method is more conservative than Tukey's method. In other words, the simultaneous confidence intervals based on the Tukey method are shorter.

The Tukey Method

- ▶ In the coagulation study $N = 24$, $k = 4$ so the 5% critical value of the Studentized range distribution is obtained using the the inverse CDF function `qtukey()` for this distribution. Tukey.
↑ gives crit. value.
- ▶ The argument `lower.tail=FALSE` is used so we obtain the upper percentile of the distribution (i.e., the value of x such that $P(X > x) = 0.05$).

```
qtukey(p = .05, nmeans = 4, df = 20, lower.tail = FALSE)
```

```
## [1] 3.958293
```

The Tukey Method

Let's obtain the Tukey p-value and confidence interval for $\mu_B - \mu_A$. The observed value of the test statistic is:

$$q^{obs} = \sqrt{2}|t_{AB}|,$$

where

$$t_{AB} = \frac{\bar{y}_A - \bar{y}_B}{\hat{\sigma} \sqrt{1/n_A + 1/n_B}}.$$

```
(sqrt(2)*(66-61))/(sqrt(5.6)*sqrt(1/6+1/6))
```

```
## [1] 5.175492
```

- The Tukey procedure uses the Studentized range dist. to assess p-value and CI.

The Tukey Method

The p-value

how extreme
on Studentized
range list.?

$$P(q_{4,20} > q^{obs}) = 0.008$$

is then obtained using the CDF of the Studentized range distribution

```
1-ptukey(q = sqrt(2)*5/sqrt(2*5.6/6), nmeans = 4, df = 20)
```

```
## [1] 0.007797788
```

↑
 q^{obs}

↑
means

↑
N-k.

21
5.17

The Tukey Method

The 95% limits of the Tukey confidence interval for $\mu_B - \mu_A$ is

```
tuk.crit <- qtukey(p=.05,nmeans=4,df=20,lower.tail=FALSE)
#lower limit
round(5-(1/sqrt(2))*tuk.crit*sqrt(5.6)*sqrt(1/6+1/6),2)
```

```
## [1] 1.18
```

est - error

```
#upper limit
```

```
round(5+(1/sqrt(2))*tuk.crit*sqrt(5.6)*sqrt(1/6+1/6),2)
```

```
## [1] 8.82
```

est + error

The Tukey Method

$$\begin{aligned} \text{CI width} &= \text{error} \\ &= \text{crit.value} \times \text{S.e} \end{aligned}$$

The width of the Tukey confidence interval for $\mu_B - \mu_A$ is

```
round((1/sqrt(2))*tuk.crit*sqrt(5.6)*sqrt(1/6+1/6),2)
```

```
## [1] 3.82
```

The width of Bonferroni $\mu_B - \mu_A$ is

```
round(qt(p = 1-0.004,df = 20)*sqrt(5.6)*sqrt(1/6+1/6),2)
```

```
## [1] 4.02
```

The Tukey Method

- ▶ This shows that the Tukey confidence interval is shorter than Bonferroni confidence intervals.
- ▶ The command `TukeyHSD()` can be used to obtain all the Tukey confidence intervals and p-values for an ANOVA.

The Tukey Method

```
TukeyHSD(aov(y~diets,data=tab0401))
```

```
round(TukeyHSD(aov(y~diets,data=tab0401))$diets,2)
```

##		diff	lwr	upr	p adj	
##	B-A	5	1.18	8.82	0.01	*
##	C-A	7	3.18	10.82	0.00	*
##	D-A	0	-3.82	3.82	1.00	
##	C-B	2	-1.82	5.82	0.48	
##	D-B	-5	-8.82	-1.18	0.01	*
##	D-C	-7	-10.82	-3.18	0.00	*

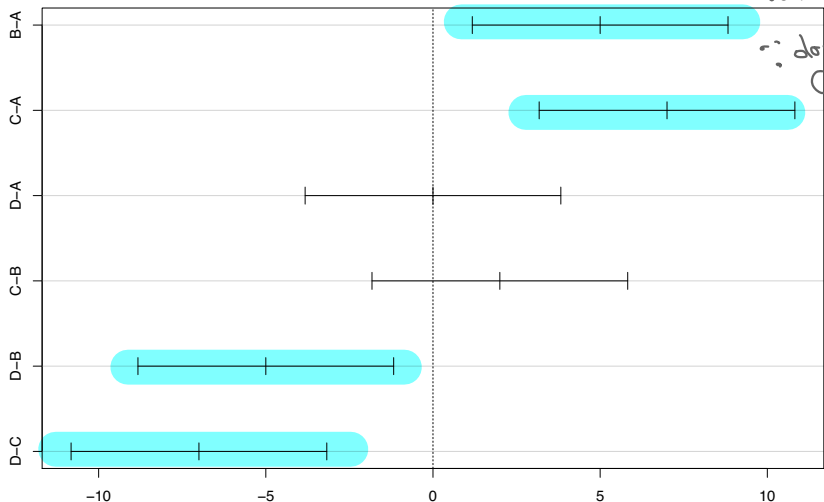
↑ does not contain 0.

The Tukey Method

```
plot(TukeyHSD(aov(y~diets,data=tab0401)))
```

95% family-wise confidence level

Significant
at $\alpha=0.05$



\therefore don't
contain
0.

Differences in mean levels of diets