BMEG 802 – Advanced Biomedical Experimental Design and Analysis

Mixed Analysis of Variance (ANOVA)

Joshua G. A. Cashaback, PhD

Recap

- 1-Way Repeated Measures (“within” subjects) Design
  - Repeated Meaures ANOVA
    - Greenhouse-Geisser Corrections
  - Multivariate Approach
  - Friedman Test
- 2-Way Repeated Measures
  - Repeated Meaures ANOVA
    - Greenhouse-Geisser Corrections
  - Multivariate Approach

Today

- Mixed (Between-Within) Design
  - 2 or more groups
  - 2 or more repeated measures

Mixed ANOVA

- combines within and between ANOVA
- Same concepts and principles apply
  - assumptions
  - interactions, main effects
    - follow-up mean comparisons

Examples of a Mixed Design

Effectiveness of a drug (between level) over time (within level)

- e.g., Between (group 1: Moderna vs group 2: Pfizer) how effective are different vaccines over time (1 month, 2 month, etc)?

General Linear Model (GLM)

Full Model: $Y_{ij} = \mu + \alpha_j + \beta_k + \pi_{i/j} + (\alpha\beta)_{jk} + (\beta\pi)_{ki/j} + \epsilon_{ijk}$

Effect of Between Factor

Effect of Within Factor

Effect of Subjects

see Maxwell, Delaney, Kelley for details (restricted models, error, df, etc.)

Assumptions of Mixed ANOVA

- same assumptions as between and within designs
- we have to do the typical tests to check for:
  - normality
  - homogeneous of variance (sphericity)
    - Greenhouse-Geisser adjustments for within terms

Three Group Example in R

Let's determine if there is a main effect of age (Young vs Old) and physical therapy treatment over time. The dependent measure is pain out of 10 (lower values represent less pain).

Group	Treatment 1	Treatment 2	Treatment 3
O	8	5	3
O	7	6	6
O	8	7	6
O	7	5	4
Y	6	5	2
Y	5	5	4
Y	5	4	3
Y	6	3	2

Setting up the Data

```
pain <- c(8,5,3,7,6,6,8,7,6,7,5,4,6,5,2,5,5,4,5,4,3,6,3,2)
treatment <- factor(rep(c(1,2,3),8))
age <- factor(c(rep('O',12),rep('Y',12)))
subject <- factor(c(rep(1,3),rep(2,3),rep(3,3),
                    rep(4,3),rep(5,3), rep(6,3),rep(7,3),rep(8,3)))
mydata <- data.frame(subject, pain, treatment, age)
```

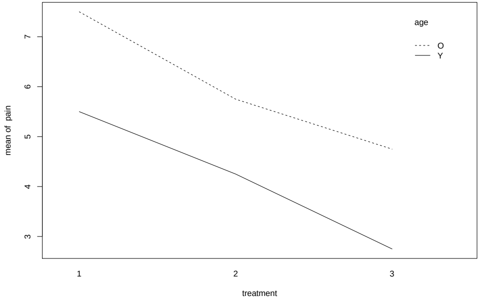
Setting up the Data

```
mydata
```

```
##    subject pain treatment age
## 1        1    8         1   O
## 2        1    5         2   O
## 3        1    3         3   O
## 4        2    7         1   O
## 5        2    6         2   O
## 6        2    6         3   O
## 7        3    8         1   O
## 8        3    7         2   O
## 9        3    6         3   O
## 10       4    7         1   O
## 11       4    5         2   O
## 12       4    4         3   O
## 13       5    6         1   Y
## 14       5    5         2   Y
## 15       5    2         3   Y
## 16       6    5         1   Y
## 17       6    5         2   Y
## 18       6    4         3   Y
## 19       7    5         1   Y
## 20       7    4         2   Y
## 21       7    3         3   Y
## 22       8    6         1   Y
## 23       8    3         2   Y
## 24       8    2         3   Y
```

Plot Data

```
interaction.plot(treatment, age, pain)
```



Install Mixed ANOVA Package

install.package("rstatix")

```
library(rstatix)
```

```
##
## Attaching package: 'rstatix'
## The following object is masked from 'package:stats':
##
##     filter
```

Mixed ANOVA Output

```r
res.aov <- anova_test(pain ~ age * treatment + Error(subject/(treatment)), data = mydata, type = 3, effect.size = "pes")
res.aov # note subject divided by within factor # pes is partial eta squared
```

```
## ANOVA Table (type III tests)
##
## $ANOVA
##          Effect DFn DFd      F       p p<.05   pes
## 1           age   1   6 15.783 0.00700     * 0.725
## 2     treatment   2  12 19.500 0.00017     * 0.765
## 3 age:treatment   2  12  0.214 0.81000       0.034
##
## $`Mauchly's Test for Sphericity`
##          Effect    W     p p<.05
## 1     treatment 0.54 0.214
## 2 age:treatment 0.54 0.214
##
## $`Sphericity Corrections`
##          Effect   GGe      DF[GG] p[GG] p[GG]<.05   HFe      DF[HF]    p[HF]
## 1     treatment 0.685 1.37, 8.22 0.001         * 0.819 1.64, 9.83 0.000548
## 2 age:treatment 0.685 1.37, 8.22 0.730           0.819 1.64, 9.83 0.769000
##   p[HF]<.05
## 1         *
## 2
```

Mixed ANOVA - GG Corrections

anova_test reports the adjusted df, but not the F-statistic. So lets do that ourselves.

```
# Main effect of Treatment
pval = 0.001
df1_adj = 1.37
df2_adj = 8.22
Fscore = qf(1 - pval, df1=df1_adj, df2=df2_adj) # chi-square function
Fscore
```

```
## [1] 21.24584
```

```
# Interaction between Age and Treatment
pval = 0.73
df1_adj = 1.37
df2_adj = 8.22
Fscore = qf(1 - pval, df1=df1_adj, df2=df2_adj) # chi-square function
Fscore
```

```
## [1] 0.2141381
```

Note: for 2 levels of within factor there is no sphericity to correct for since it is 1D (i.e., only variance along a line; not a circle, sphere, hypersphere!)

Mixed ANOVA Interpretation

- Main Effect of Age [F(1,6) $= 15.8$, p $= 0.007$, $\eta_p^2 = 0.725$] and Treatment [F(1.37, 8.22) $= 21.2$, p $= 0.001$, $\eta_p^2 = 0.765$].
- There was not a significant interaction between Age and Treatment [F(1.37,8.22) $= 0.214$, p $= 0.730$, $\eta_p^2 = 0.034$]
- Perform followup mean comparisons & effect sizes for the Main Effects ONLY
  - same rules apply on the order to examine interaction or main effects as in 2-way between ANOVA and 2-way within ANOVA designs.

Mixed ANOVA Effect Size

- Here we outputted η_p^2 (one of the options of this particular R package)
- Can also calculate ω_p^2
    - not in this package (can be performed by hand or some other package)
    - Bakeman, R. (2005). Recommended effect size statistics for repeated measures designs. Behavior research methods, 37(3), 379-384.
    - I'll leave it to you to explore further.

Repeated Measures ANOVA - Sphercity Violations

Repeated Measures are very sensitive to Sphericity Violations

1. Greenhouse-Geisser Corrections (we did this)
2. Multivariate Approach

- not going to do today, but just so you are aware this is an alternative option.

Organize Data (2x3 design)

Let's quickly split our data by

```
O1 = c(pain[1],pain[4],pain[7],pain[10])  # old, treatment 1
O2 = c(pain[2],pain[5],pain[8],pain[11])
O3 = c(pain[3],pain[6],pain[9],pain[12])
Y1 = c(pain[13],pain[16],pain[19],pain[22])
Y2 = c(pain[14],pain[17],pain[20],pain[23])
Y3 = c(pain[15],pain[18],pain[21],pain[24]) # young, treatment 3
```

Organize Data - Main Effects of Age

Let's quickly split our data by age

```
Old = c(O1, O2, O3)  # old
Young = c(Y1, Y2, Y3)  # young
```

Testing normalility for Main Effect of Age

```r
shapiro.test(Old)$p.value
```

```
## [1] 0.5130488
```

```r
shapiro.test(Young)$p.value
```

```
## [1] 0.1873705
```

Data are normally distributed ($p > 0.05$)

Follow up Mean Comparisons for Main Effect of Age

```r
pOvY = t.test(Old, Young, paired = FALSE, alternative = "two.sided")$p.value
pOvY
```

```
## [1] 0.005896452
```

There is significantly more pain in the old than young groups ($p = 0.006$)

NOTE: here we use a between group mean comparison (paired = FALSE)

Sample Effect Size for Main Effect of Age

install.packages("effsize")

```
library(effsize)
cohen.d(Old, Young, paired = FALSE)$estimate #
```

```
## [1] 1.245505
```

Organize Data - Main Effects of Treatment

Let's quickly split our data by treatment

```
treat1 = c(O1, Y1)  # time 1
treat2 = c(O2, Y2)  # time 2
treat3 = c(O3, Y3)  # time 3
```

Very important to keep Old and Young in same order, since we will be looking at the PAIRED differences

Testing normalility for Main Effect of Treatment

Performed on the PAIRED differences

```r
shapiro.test(treat2-treat1)$p.value #
```

```
## [1] 0.1199348
```

```r
shapiro.test(treat3-treat1)$p.value #
```

```
## [1] 0.4283399
```

```r
shapiro.test(treat3-treat1)$p.value #
```

```
## [1] 0.4283399
```

Data are normally distributed ($p > 0.05$)

Follow up Mean Comparisons for Main Effect of Treatment

```
p1v2 = t.test(treat1, treat2, paired = TRUE, alternative = "two.sided")$p.value #
p1v3 = t.test(treat1, treat3, paired = TRUE, alternative = "two.sided")$p.value #
p2v3 = t.test(treat2, treat3, paired = TRUE, alternative = "two.sided")$p.value #
pvals = c(p1v2, p1v3, p2v3)
pvals_holm = p.adjust(pvals, method = "holm", n = length(pvals))
sprintf("%.5f", pvals_holm) #outputs in decimal (not scientific notation)
```

```
## [1] "0.01053" "0.00365" "0.01053"
```

There is a reduction in pain between treatments 1 and 2 ($p = 0.011$), treatments 1 and 3 ($p < 0.004$), and treatment 2 and 3 ($p = 0.011$)

Remember, treatment is a within factor (paired = TRUE)

Sample Effect Size for Main Effect of Treatment

```r
library(effsize)
cohen.d(treat1, treat2, paired = TRUE)$estimate #
```

```
## [1] 1.25499
```

```r
cohen.d(treat1, treat3, paired = TRUE)$estimate #
```

```
## [1] 1.931982
```

```r
cohen.d(treat2, treat3, paired = TRUE)$estimate #
```

```
## [1] 0.8185863
```

Summary of Mixed ANOVA

- We found a significant main effects of Age [$F(1,6) = 15.8$, $p = 0.007$, $\eta_p = 0.725$] and Treatment [$F(1.37, 8.22) = 21.2$, $p = 0.001$, $\eta_p^2 = 0.765$].
- The elderly had significantly more pain the younger patients ($p = 0.006$, $d = 1.26$).
- There was a reduction in pain between treatments 1 and 2 ($p = 0.011$, $d = 1.25$), treatments 1 and 3 ($p = 0.004$, $d = 1.93$), and treatment 2 and 3 ($p = 0.011$, $d = 0.82$).

Mixed ANOVA Interactions

- We did not have a significant interaction between age and treatment
- But what is the procedure if we had found one?
  - As with the previous 2-way ANOVA, we have to decide which 'direction' to look
    - Age
    - Treatment
  - Let's go through both as an example, but remember you only pick one direction if you find a significant interaction (typically the between effect in a mixed design).

Mixed ANOVA Interactions - Normality

```r
shapiro.test(O1)$p.value
```

```
## [1] 0.02385679
```

```r
shapiro.test(O2)$p.value
```

```
## [1] 0.2724532
```

```r
shapiro.test(O3)$p.value
```

```
## [1] 0.2242305
```

```r
shapiro.test(Y1)$p.value
```

```
## [1] 0.02385679
```

```r
shapiro.test(Y2)$p.value
```

```
## [1] 0.2724532
```

```r
shapiro.test(Y3)$p.value
```

```
## [1] 0.2724532
```

Data are normally distributed ($p > 0.05$)

Mixed ANOVA Interactions (Examining Age) - Mean Comparisons

```
p1v2 = t.test(O1, Y1, paired = FALSE, alternative = "two.sided")$p.value #
p1v3 = t.test(O2, Y2, paired = FALSE, alternative = "two.sided")$p.value #
p2v3 = t.test(O3, Y3, paired = FALSE, alternative = "two.sided")$p.value #
pvals = c(p1v2, p1v3, p2v3)
pvals_holm = p.adjust(pvals, method = "holm", n = length(pvals))
sprintf("%.5f", pvals_holm) #outputs in decimal (not scientific notation)
```

```
## [1] "0.00814" "0.13722" "0.13722"
```

note: we found a significant different between young and old at treatment 1, but we did not actually have permission to examine this! remember, we are just practicing the prodecure.

Mixed ANOVA Interactions (Examining Age) - Effect Size

```r
library(effsize)
cohen.d(O1, Y1, paired = FALSE)$estimate #
```

```
## [1] 3.464102
```

```r
cohen.d(O2, Y2, paired = FALSE)$estimate #
```

```
## [1] 1.566699
```

```r
cohen.d(O3, Y3, paired = FALSE)$estimate #
```

```
## [1] 1.589439
```

Mixed ANOVA Interactions (Examining Treatment) - Mean Comparisons

```
pO1vO2 = t.test(O1, O2, paired = TRUE, alternative = "two.sided")$p.value
pO1vO3 = t.test(O1, O3, paired = TRUE, alternative = "two.sided")$p.value
pO2vO3 = t.test(O2, O3, paired = TRUE, alternative = "two.sided")$p.value
pY1vY2 = t.test(Y1, Y2, paired = TRUE, alternative = "two.sided")$p.value
pY1vY3 = t.test(Y1, Y3, paired = TRUE, alternative = "two.sided")$p.value
pY2vY3 = t.test(Y2, Y3, paired = TRUE, alternative = "two.sided")$p.value
pvals = c(pO1vO2, pO1vO3, pO2vO3, pY1vY2, pY1vY3, pY2vY3)
pvals_holm = p.adjust(pvals, method = "holm", n = length(pvals))
sprintf("%.5f", pvals_holm) #outputs in decimal (not scientific notation)
```

```
## [1] "0.21049" "0.21049" "0.21049" "0.21049" "0.21049" "0.21049"
```

Mixed ANOVA Interactions (Examining Treatment) - Effect Size

```r
library(effsize)
cohen.d(O1, O2, paired = TRUE)$estimate #
```

```
## [1] 2.160364
```

```r
cohen.d(O1, O3, paired = TRUE)$estimate #
```

```
## [1] 2.486706
```

```r
cohen.d(O2, O3, paired = TRUE)$estimate #
```

```
## [1] 0.6235665
```

```r
cohen.d(Y1, Y2, paired = TRUE)$estimate #
```

```
## [1] 1.602739
```

```r
cohen.d(Y1, Y3, paired = TRUE)$estimate #
```

```
## [1] 3.578086
```

```r
cohen.d(Y2, Y3, paired = TRUE)$estimate #
```

```
## [1] 1.566699
```

Power Analysis on ANOVA

install.packages("WebPower")

```r
library(WebPower)
```

```
## Loading required package: MASS

##
## Attaching package: 'MASS'

## The following object is masked from 'package:rstatix':
##
##     select

## Loading required package: lme4

## Loading required package: Matrix

## Loading required package: lavaan

## This is lavaan 0.6-7
```

Power Analysis on ANOVA

install.packages("WebPower")

```
library(WebPower)
# n=sub, ng=#ofgroups, nm=#ofmeasurements, nscor=sphericity(1=perfect)
#type "0" between-effect; "1" within-effect; and "2" interaction effect
wp.rmanova(n = NULL, ng = 2, nm = 3, f = .4, nscor = 1,
           alpha = 0.05, power = 0.8, type = 2)
```

```
## Repeated-measures ANOVA analysis
##
##             n   f ng nm nscor alpha power
##      61.75222 0.4  2  3     1  0.05   0.8
##
## NOTE: Power analysis for interaction-effect test
## URL: http://psychstat.org/rmanova
```

We need 62 participants per group for a sufficiently powered.

Next Week

- ANCOVA
  - Similar to ANOVA
  - regressing out covariates (e.g., age)