

Two factorial analysis of variance

Two factorial analysis of variance without interaction

Two factorial analysis of variance

- Question:** efficacy testing of an antidepressive drug
dependent on
Factor 1: Dose
Factor 2: Sex
- Sample:** A total of 30 subjects
- Design:** Treatment of 5 male and 5 female subjects
with low dose (Dose 1, D1) and high dose (Dose 2
D2) with Placebo (P)
- Model:** **Degree of depression** is normally distributed with
equal variances in the groups

Raw Data (Bortz, 1993)

	Male					Female				
Placebo	22	25	22	21	22	18	19	17	21	19
Dose 1	16	16	16	15	15	19	20	17	16	16
Dose 2	13	12	12	13	12	16	14	16	13	14

Two factorial analysis of variance without interaction

$$X_{ijk} = \mu_{ij} + \varepsilon_{ijk} = \mu + \alpha_i + \beta_j + \varepsilon_{ijk}$$

$i = 1, \dots, I$ (Factor 1: Treatment, $I = 3$)

$j = 1, \dots, J$ (Factor 2: Sex, $J = 2$)

$k = 1, \dots, K$ (subject within group)

$$\sum_{i=1}^I \alpha_i = \sum_{j=1}^J \beta_j = 0$$

Note: We only treat the case with equal groups sizes.

Each value is from a different subject! (i.e. the ε_{ijk} are independent!)

Explanation of the parameters

X_{ijk} : Value k in the group with factor level i and j

μ : Total mean

α_i : Deviation group mean i factor 1 vs total mean

β_j : Deviation group mean j factor 2 vs total mean

ε_{ijk} : Deviation of x_{ijk} from the mean $\mu + \alpha_i + \beta_j$

ε_{ijk} are normally distributed

with mean 0

and fixed variance σ^2

and independent

Exercise

$$X_{ijk} = \mu_{ij} + \varepsilon_{ijk} = \mu + \alpha_i + \beta_j + \varepsilon_{ijk}$$

$$\sum_{i=1}^I \alpha_i = \sum_{j=1}^J \beta_j = 0$$

The middle term includes $J \bullet K = 3 \bullet 2 = 6$ free parameters as there are no restrictions on the parameters μ_{ij} .

How many free parameters includes the right term?

Give an interpretation why the right term contains less parameters. In which sense is thus the right term a special case of the middle term?

Parameter estimation in the two-factorial analysis of variance without interaction

The parameters in the equation

$$X_{ijk} = \mu + \alpha_i + \beta_j + \varepsilon_{ijk} \quad i = 1 \dots I, j = 1 \dots J, k = 1 \dots K$$

are estimated by

$$\hat{\mu} = \bar{X}, \quad \hat{\alpha}_i = \bar{X}_i - \bar{X}, \quad \hat{\beta}_j = \bar{X}_j - \bar{X}$$

Parameter estimation in the two-factorial analysis of variance without interaction

Now we replace the theoretical parameters by their estimates to obtain an estimate of the error term.

$$x_{ijk} = \bar{x} + (\bar{x}_i - \bar{x}) + (\bar{x}_j - \bar{x}) + \hat{\varepsilon}_{ijk}$$

$$x_{ijk} - (\bar{x} + (\bar{x}_i - \bar{x}) + (\bar{x}_j - \bar{x})) = \hat{\varepsilon}_{ijk}$$

$$x_{ijk} - \bar{x} - \bar{x}_i + \bar{x} - \bar{x}_j + \bar{x} = \hat{\varepsilon}_{ijk}$$

$$x_{ijk} - \bar{x}_i - \bar{x}_j + \bar{x} = \hat{\varepsilon}_{ijk}$$

$$x_{ijk} = \mu + \alpha_i + \beta_j + \varepsilon_{ijk} \quad \hat{\mu} = \bar{x}, \quad \hat{\alpha}_i = \bar{x}_i - \bar{x}, \quad \hat{\beta}_j = \bar{x}_j - \bar{x} \quad 8$$

The split of the total sum of squares

Total sum of
squares: SSQ_{total}

Sum of squares
between groups
(Factor 1):
 $SSQ_{\text{between, Factor 1}}$

Sum of squares
between groups
(Factor 2):
 $SSQ_{\text{between, Factor 2}}$

$$\sum_{i=1}^I \sum_{j=1}^J \sum_{k=1}^K [x_{ijk} - \bar{x}]^2 = \sum_{i=1}^I JK (\bar{x}_i - \bar{x})^2 + \sum_{j=1}^J IK (\bar{x}_j - \bar{x})^2 + \sum_{i=1}^I \sum_{j=1}^J \sum_{k=1}^K (x_{ijk} - \bar{x}_i - \bar{x}_j + \bar{x})^2$$

Sum of squares within groups: SSQ_{within}

Two-factorial analysis of variance: tests of significance

$$SSQ_{\text{tot}} = SSQ_{\text{between (Factor 1)}} + SSQ_{\text{between(Faktor 2)}} + SSQ_{\text{within}}$$

$$MSQ_{\text{between (Factor 1)}} = 1 / (I-1) \cdot SSQ_{\text{between (Factor 1)}}$$

$$MSQ_{\text{between (Factor 2)}} = 1 / (J-1) \cdot SSQ_{\text{between (Factor2)}}$$

$$MSQ_{\text{within}} = 1 / [N-I-J+1] \cdot SSQ_{\text{within}}$$

$$F_{\text{Factor1}} = MSQ_{\text{between (Factor 1)}} / MSQ_{\text{within}} \quad DF = I-1, [N-I-J+1]$$

$$F_{\text{Factor2}} = MSQ_{\text{between(Factor 2)}} / MSQ_{\text{within}} \quad DF = J-1, [N-I-J+1]$$

Example: Sum of squares

Sums of squares between the treatments

$$SSQ_{\text{Treatments}} = 253.4 \quad MSQ_{\text{Treatments}} = 1/2 \bullet 253.4 = 126.7$$

Sums of squares between the sexes

$$SSQ_{\text{sex}} = 0.300 \quad MSQ_{\text{sex}} = 1/1 \bullet 0.300 = 0.300$$

Sums of squares „error“

$$SSQ_{\text{error}} = 95.00 \quad MSQ_{\text{error}} = 1/26 \bullet 95.00 = 3.654$$

Example: Test of Significance

Treatments

$$F = 126.7 / 3.654 \approx 34.7 \Rightarrow p < 0.001$$

Sex

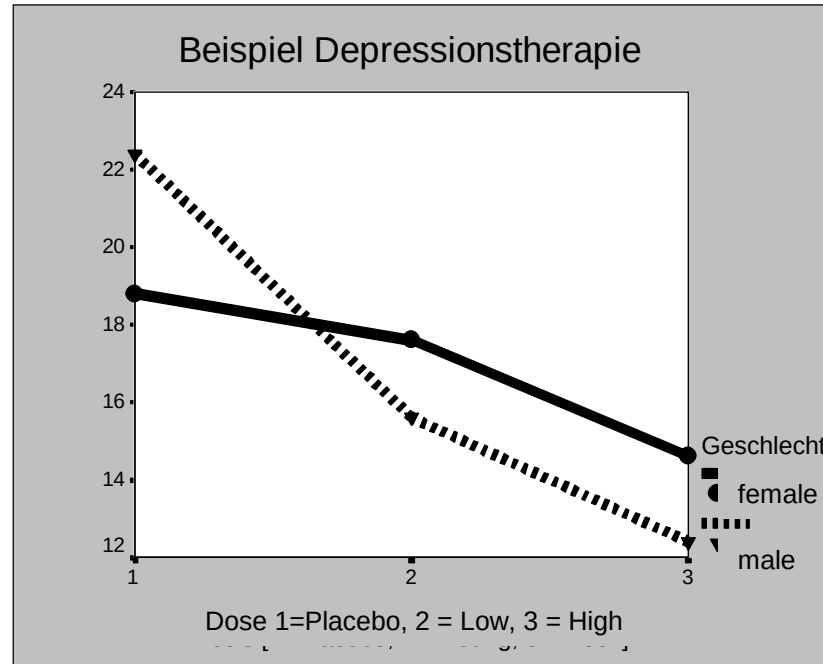
$$F = 0.3 / 3.654 \approx 0.082 \Rightarrow p = 0.77$$

There was a significant difference ($\alpha = 5\%$) between the treatments.

There was no significant difference between the sexes.

Two factorial analysis of variance with interaction

Two-factorial analysis of variance with interaction



The effect of dose seems to be different in female and male subjects. This is ignored in the model without interaction!

Two-factorial analysis of variance with interaction

$$X_{ijk} = \mu_{ij} + \varepsilon_{ijk} = \mu + \alpha_i + \beta_j + \gamma_{ij} + \varepsilon_{ijk}$$

$$i = 1 \dots I, \quad j = 1 \dots J, \quad k = 1 \dots K$$

$$\sum_{i=1}^I \alpha_i = \sum_{j=1}^J \beta_j = \sum_{i=1}^I \gamma_{ij} = \sum_{j=1}^J \gamma_{ij} = 0$$

γ_{ij} : Deviance mean of group ij from $(\mu + \alpha_i + \beta_j)$

ε_{ijk} : Deviance single value x_{ijk} from the mean μ_{ij}

ε_{ijk} "as usual: normally distributed, mean 0, constant variance σ^2
and independent (from different subjects)!"

All other notation does not change.

Exercise

$$X_{ijk} = \mu_{ij} + \varepsilon_{ijk} = \mu + \alpha_i + \beta_j + \gamma_{ij} + \varepsilon_{ijk}$$

$$\sum_{i=1}^I \alpha_i = \sum_{j=1}^J \beta_j = \sum_{i=1}^I \gamma_{ij} = \sum_{j=1}^J \gamma_{ij} = 0$$

The middle term includes $J \bullet K = 3 \bullet 2 = 6$ free parameters (see exercise sheet 5).

How many free parameters includes the right term?

Two factorial analysis of variance with interaction: Parameter estimation

The unknown parameters in the model equation with interaction

$$X_{ijk} = \mu + \alpha_i + \beta_j + \gamma_{ij} + \varepsilon_{ijk} \quad i = 1 \dots I, j = 1 \dots J, k = 1 \dots K$$

are estimated identically to the case without interaction!

Except for the interaction term.

This term is estimated according to the formula

$$\hat{\gamma}_{ij} = \bar{X}_{ij} - \bar{X}_i - \bar{X}_j + \bar{X}$$

Two factorial analysis of variance with interaction: Parameter estimation

Now we replace the theoretical parameters by their estimates to obtain an estimate of the error term. This estimate is much more simple than the one without interaction.

$$x_{ijk} = \bar{x} + (\bar{x}_i - \bar{x}) + (\bar{x}_j - \bar{x}) + \bar{x}_{ij} - \bar{x}_i - \bar{x}_j + \bar{x} + \hat{\varepsilon}_{ijk}$$

$$x_{ijk} = \bar{x}_{ij} + \hat{\varepsilon}_{ijk}$$

$$x_{ijk} - \bar{x}_{ij} = \hat{\varepsilon}_{ijk}$$

$$x_{ijk} = \mu + \alpha_i + \beta_j + \gamma_{ij} + \varepsilon_{ijk}$$

$$\hat{\mu} = \bar{x}, \quad \hat{\alpha}_i = \bar{x}_i - \bar{x}, \quad \hat{\beta}_j = \bar{x}_j - \bar{x}, \quad \hat{\gamma}_{ij} = \bar{x}_{ij} - \bar{x}_i - \bar{x}_j + \bar{x}$$

Two factorial analysis of variance with interactions

The split of the sums of squares

For each combination of the factors „Treatment“ (P, D1, D2) and „sex“ (m/f) we have multiple measurements. This allows to split the sum of squares further:

→

$$\begin{aligned} \text{SSQ}_{\text{total}} &= \text{SSQ}_{\text{Treatment}} + \text{SSQ}_{\text{sex}} + \text{SSQ}_{\text{Remaining}} \\ &= \text{SSQ}_{\text{Treatment}} + \text{SSQ}_{\text{sex}} \\ &\quad + \text{SSQ}_{\text{Interaction Treatment} \bullet \text{Sex}} + \text{SSQ}_{\text{error}} \end{aligned}$$

Two factorial analysis of variance with interactions

The split of the sums of squares

$$\begin{aligned} & \sum_{i=1}^I \sum_{j=1}^J \sum_{k=1}^K [x_{ijk} - \bar{x}]^2 \\ &= \\ & \sum_{i=1}^I J \bullet K (\bar{x}_i - \bar{x})^2 + \sum_{j=1}^J I \bullet K (\bar{x}_j - \bar{x})^2 + \sum_{i=1}^I \sum_{j=1}^J K (\bar{x}_{ij} - \bar{x}_i - \bar{x}_j + \bar{x})^2 \\ &+ \\ & \sum_{i=1}^I \sum_{j=1}^J \sum_{k=1}^K (x_{ijk} - \bar{x}_{ij})^2 \end{aligned}$$

Two factorial analysis of variance with interactions

Tests of significance

$$SSQ_{\text{total}} = SSQ_{\text{Treatment}} + SSQ_{\text{sex}} + SSQ_{\text{interaction}} + SSQ_{\text{error}}$$

$$MSQ_{\text{between (Factor1)}} = 1 / (I - 1) \bullet SSQ_{\text{between (Factor 1)}}$$

$$MSQ_{\text{between (Factor2)}} = 1 / (J - 1) \bullet SSQ_{\text{between (Factor2)}}$$

$$MSQ_{\text{interaction}} = 1 / [(I - 1) \bullet (J - 1)] \bullet SSQ_{\text{interaction}}$$

$$MSQ_{\text{error}} = 1 / [I \bullet J \bullet (K - 1)] \bullet SSQ_{\text{error}}$$

$$F_{\text{Factor 1}} = MSQ_{\text{(between (Factor 1))}} / MSQ_{\text{error}} \quad DF = I - 1, \quad I \bullet J \bullet (K - 1)$$

$$F_{\text{Factor 2}} = MSQ_{\text{(between(Factor 2))}} / MSQ_{\text{error}} \quad DF = J - 1, \quad I \bullet J \bullet (K - 1)$$

$$F_{\text{interaction}} = MSQ_{\text{interaction}} / MSQ_{\text{error}} \quad DF = (I - 1) \bullet (J - 1), I \bullet J \bullet (K - 1)$$

Example: sums of squares

Between Treatments

$$SSQ_{\text{Dosis}} = 253.4 \quad MSQ_{\text{Dosis}} = 1/2 \cdot 253.4 = 126.7$$

Between sexes

$$SSQ_{\text{Geschlecht}} = 0.3 \quad MSQ_{\text{Geschlecht}} = 1/1 \cdot 0.3 = 0.3$$

Interaction Treatment Sex

$$SSQ_{\text{Wewi}} = 54.2 \quad MSQ_{\text{Wewi}} = 1/2 \cdot 54.2 = 27.1$$

Error

$$SSQ_{\text{Fehler}} = 40.8 \quad MSQ_{\text{Fehler}} = 1/24 \cdot 40.8 = 1.7$$

Example: Test of Significance

Treatment

$$F = 126.7 / 1.7 \approx 74.5 \Rightarrow p < 0.001$$

Sex

$$F = 0.3 / 1.7 \approx 0.18 \Rightarrow p = 0.68$$

Treatment • Sex

$$F = 27.1 / 1.7 \approx 15.9 \Rightarrow p < 0.001$$

Example: Interpretation

The significant interaction demonstrates that we have to determine the dose – response effect separately for male and female subjects.

The estimated parameter of dose and sex do not change compared to the analysis without interaction. However, the interpretation changes: In the model **without interaction** the dose effect is assumed **equal** for both sexes. In the model **with interaction**, the **average** dose effect of male and female subjects is estimated.

There is a „convention“ to leave a factor in a model, if an interaction with this factor is significant even if the factor itself is not significant. So we will leave sex in our final model.

The case of only one observation per factor combination

The example of Bortz - revised

	Male	Female
Placebo	22.4	18.8
Dose 1	15.6	17.6
Dose 2	12.4	14.6

Revised Example: The split of the total sum of squares

Total sum of
squares: SSQ_{total}

Sum of squares
between groups
(Factor 1):
 $SSQ_{\text{between, Factor 1}}$

Sum of squares
between groups
(Factor 2):
 $SSQ_{\text{between, Factor 2}}$

$$\sum_{i=1}^I \sum_{j=1}^J \sum_{k=1}^K [x_{ijk} - \bar{x}]^2 = \sum_{i=1}^I JK (\bar{x}_i - \bar{x})^2 + \sum_{j=1}^J IK (\bar{x}_j - \bar{x})^2 + \sum_{i=1}^I \sum_{j=1}^J \sum_{k=1}^K (x_{ijk} - \bar{x}_i - \bar{x}_j + \bar{x})^2$$

Sum of squares within groups: SSQ_{within}

Revised Example: K = 1

Total sum of
squares: SSQ_{total}

Sum of squares
between groups
(Factor 1):
 $SSQ_{\text{between, Factor 1}}$

Sum of squares
between groups
(Factor 2):
 $SSQ_{\text{between, Factor 2}}$

$$\sum_{i=1}^I \sum_{j=1}^J \sum_{k=1}^K [x_{ijk} - \bar{x}]^2 = \sum_{i=1}^I JK(\bar{x}_i - \bar{x})^2 + \sum_{j=1}^J IK(\bar{x}_j - \bar{x})^2 \\ + \sum_{i=1}^I \sum_{j=1}^J \sum_{k=1}^1 (x_{ijk} - \bar{x}_i - \bar{x}_j + \bar{x})^2$$

Sum of squares within groups: SSQ_{within}

Revised Example: No summation over k

Total sum of
squares: SSQ_{total}

Sum of squares
between groups
(Factor 1):
 $SSQ_{\text{between, Factor 1}}$

Sum of squares
between groups
(Factor 2):
 $SSQ_{\text{between, Factor 2}}$

$$\sum_{i=1}^I \sum_{j=1}^J \sum_{k=1}^K [x_{ijk} - \bar{x}]^2 = \sum_{i=1}^I JK(\bar{x}_i - \bar{x})^2 + \sum_{j=1}^J IK(\bar{x}_j - \bar{x})^2 \\ + \sum_{i=1}^I \sum_{j=1}^J (x_{ij} - \bar{x}_i - \bar{x}_j + \bar{x})^2$$

Sum of squares within groups: SSQ_{within}

Revised Example: $x_{ij} = \bar{x}_{ij}$

Total sum of
squares: SSQ_{total}

Sum of squares
between groups
(Factor 1):
 $SSQ_{\text{between, Factor 1}}$

Sum of squares
between groups
(Factor 2):
 $SSQ_{\text{between, Factor 2}}$

$$\sum_{i=1}^I \sum_{j=1}^J \sum_{k=1}^K [x_{ijk} - \bar{x}]^2 = \sum_{i=1}^I JK(\bar{x}_i - \bar{x})^2 + \sum_{j=1}^J IK(\bar{x}_j - \bar{x})^2 \\ + \sum_{i=1}^I \sum_{j=1}^J (\bar{x}_{ij} - \bar{x}_i - \bar{x}_j + \bar{x})^2$$

Sum of squares within groups: SSQ_{within}

The example of Bortz - revised

	Male	Female
Placebo	22.4	18.8
Dose 1	15.6	17.6
Dose 2	12.4	14.6

In the revised example the error term is identical to what we used as the interaction term in the original example
i.e.

If we only have one measurement per factor combination, we can do an anova – but without interaction term. The SSQ of the interaction term is now the error term

Revised Example: Sum of squares

Sums of squares between the treatments

$$SSQ_{\text{Treatments}} = 50.680 \quad MSQ_{\text{Treatments}} = 1/2 \bullet 50.680 = 25.340$$

Sums of squares between the sexes

$$SSQ_{\text{sex}} = 0.060 \quad MSQ_{\text{sex}} = 1/1 \bullet 0.060 = 0.060$$

Sums of squares „error“

$$SSQ_{\text{error}} = 10.840 \quad MSQ_{\text{error}} = 1/2 \bullet 10.840 = 5.420$$

Revised Example: Test of Significance

Treatments

$$F = 25.340 / 5.420 \approx 4.675 \Rightarrow p = 0.176$$

Sex

$$F = 0.060 / 5.420 \approx 0.011 \Rightarrow p = 0.926$$

No significances

A different parameterization of the model with interaction

Ignoring the 2-factorial structure

	Male					Female				
Placebo	22	25	22	21	22	18	19	17	21	19
Dose 1	16	16	16	15	15	19	20	17	16	16
Dose 2	13	12	12	13	12	16	14	16	13	14

Now we completely ignore the factorial structure. We just code 6 different groups and calculate a one-factorial analysis of variance.

(1) Placebo male, (2) Placebo female, (3) Dose 1 male, (4) Dose 1 female (5) Dose 2 male, (6) Dose 2 female

SSQ ignoring the two-factorial structure

$$SSQ_{between} = 307.9$$

$$SSQ_{within} = 40.8$$

$$SSQ_{total} = 348.7$$

SSQ with two-factorial structure in the model with interaction

Between Treatments

$$SSQ_{\text{Dosis}} = 253.4 \quad MSQ_{\text{Dosis}} = 1/2 \cdot 253.4 = 126.7$$

Between sexes

$$SSQ_{\text{Geschlecht}} = 0.3 \quad MSQ_{\text{Geschlecht}} = 1/1 \cdot 0.3 = 0.3$$

Interaction Treatment Sex

$$SSQ_{\text{Wewi}} = 54.2 \quad MSQ_{\text{Wewi}} = 1/2 \cdot 54.2 = 27.1$$

Error

$$SSQ_{\text{Fehler}} = 40.8 \quad MSQ_{\text{Fehler}} = 1/24 \cdot 40.8 = 1.7$$

Exercise: compare the numerical values of the SSQs of this sheet and of the preceding sheet

Example

$$MSQ_{between} = 307.9 / 5 = 61.580$$

$$MSQ_{within} = 40.800 / 24 = 1.7$$

$$MSQ_{total} = 348.7 / 29 = 12.24$$

$$F = 61.580 / 1.7 = 36.224$$

$$P < 0.001$$

Reconstruction of the two-factorial structure in the one-factorial model using orthogonal contrasts

(1) Placebo male, (2) Placebo female, (3) Dose 1 male, (4) Dose 1 female (5) Dose 2 male, (6) Dose 2 female

	(1)	(2)	(3)	(4)	(5)	(6)
Sex						
T1						
T2						
IA1						
IA2						
1	1	1	1	1	1	1

Reconstruction of the two-factorial structure in the one-factorial model using orthogonal contrasts

(1) Placebo male, (2) Placebo female, (3) Dose 1 male, (4) Dose 1 female (5) Dose 2 male, (6) Dose 2 female

	(1)	(2)	(3)	(4)	(5)	(6)
Sex	1	-1	1	-1	1	-1
T1						
T2						
IA1						
IA2						
1	1	1	1	1	1	1

Reconstruction of the two-factorial structure in the one-factorial model using orthogonal contrasts

(1) Placebo male, (2) Placebo female, (3) Dose 1 male, (4) Dose 1 female (5) Dose 2 male, (6) Dose 2 female

	(1)	(2)	(3)	(4)	(5)	(6)
Sex	1	-1	1	-1	1	-1
T1	2	2	-1	-1	-1	-1
T2						
IA1						
IA2						
1	1	1	1	1	1	1

Reconstruction of the two-factorial structure in the one-factorial model using orthogonal contrasts

(1) Placebo male, (2) Placebo female, (3) Dose 1 male, (4) Dose 1 female (5) Dose 2 male, (6) Dose 2 female

	(1)	(2)	(3)	(4)	(5)	(6)
Sex	1	-1	1	-1	1	-1
T1	2	2	-1	-1	-1	-1
T2	0	0	1	1	-1	-1
IA1						
IA2						
1	1	1	1	1	1	1

Reconstruction of the two-factorial structure in the one-factorial model using orthogonal contrasts

(1) Placebo male, (2) Placebo female, (3) Dose 1 male, (4) Dose 1 female (5) Dose 2 male, (6) Dose 2 female

	(1)	(2)	(3)	(4)	(5)	(6)
Sex	1	-1	1	-1	1	-1
T1	2	2	-1	-1	-1	-1
T2	0	0	1	1	-1	-1
IA1	2	-2	-1	1	-1	1
IA2	0	0	1	-1	-1	1
1	1	1	1	1	1	1

Calculation of SSQ of a contrast

(1) Definition of contrast and its value in the sample

$$c = \sum_{g=1}^G \lambda_g \mu_g, \quad \hat{c} = \sum_{g=1}^G \lambda_g \bar{x}_g$$

(2) Standard error

$$SE(\hat{c}) = \sqrt{MSQ_{error} * \sum_{g=1}^G \frac{\lambda_g^2}{n_g}}$$

(3) t-value

$$\hat{c} / SE(\hat{c}) = t, \quad df = df_{error}$$

(4) F value: $F = t^2$

Calculation of SSQ of a contrast

(5) An alternative definition of the F value

$$F = \frac{MSQ(contrast)}{MSQ(error)} = \frac{SSQ(contrast)}{MSQ(error)}$$

$$SSQ(contrast) = F \bullet MSQ(error) = \left(\frac{\hat{c}}{SE(c)} \right)^2 \bullet MSQ(error)$$

$$= \frac{\hat{c}^2}{\sum_{g=1}^G \frac{\lambda_g^2}{n_g}}$$

The model with interaction is – regarding the model sum of squares and the estimated cell means - equivalent to a model with one factor and so many factor levels as there are combinations of the original factors.

Thus the model with interaction is equivalent to a one-factorial model with $(I \cdot J)$ factor levels. Of course, the interpretation of the parameters changes in this model, the original two-factorial structure is „hidden“.

In the case of only one value per factor combination, we have to „believe“ that there is no interaction and we use the interaction sum of squares as error sum of squares. We cannot calculate the one-factor model ignoring the two-factorial structure.

Adjustment for Baseline

In a regular study, the depressivity of subjects should have been measured before the initiation of treatment (baseline). We could analyse just the differences between depressivity „ after treatment minus baseline “.

This method is not optimal as it assumes a constant effect of therapy for each baseline status. A more flexible approach assumes that the difference might vary (according to a linear function) dependent on the baseline value.



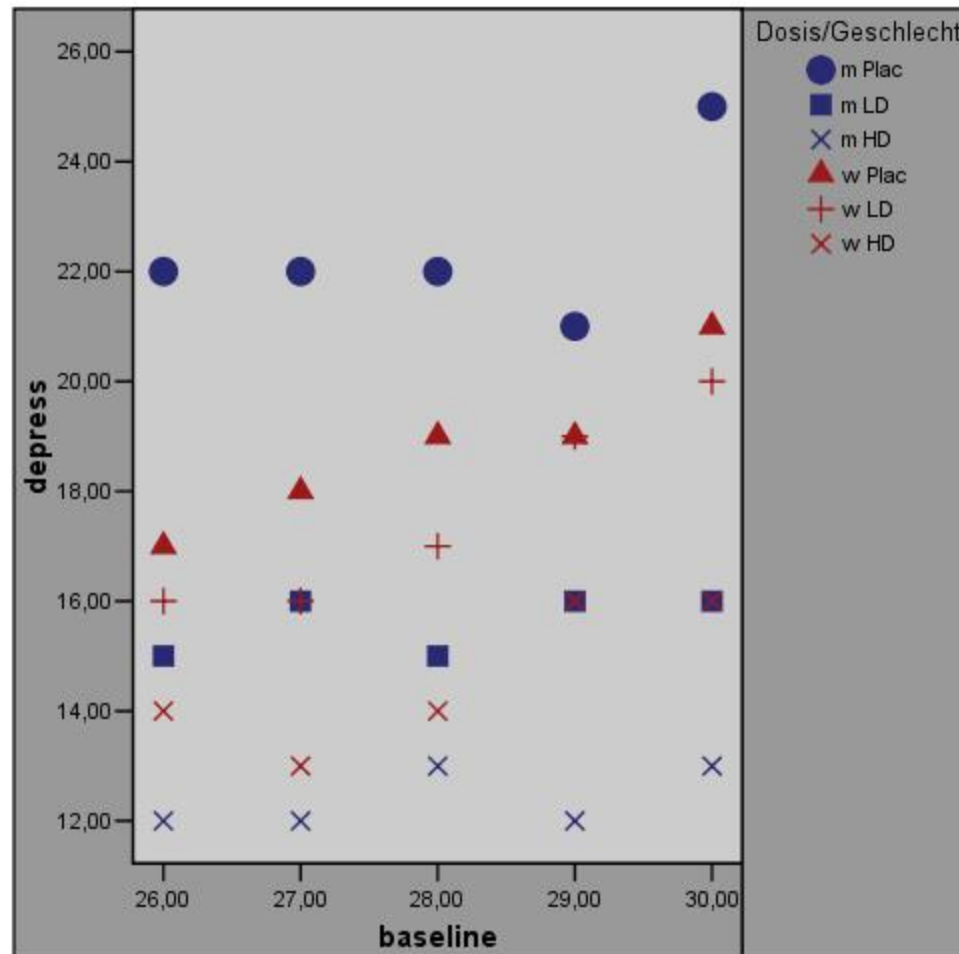
This can be done in two ways. In the field of analysis of variance we choose the analysis of covariance. In general, we could also apply the method of the General Linear Model.

Analysis of covariance

Depression study including Baseline (modified from Bortz, 1993)

	Male					Female				
Placebo	22	25	22	21	22	18	19	17	21	19
Baseline	27	30	26	29	28	27	29	26	30	28
Low Dose	16	16	16	15	15	19	20	17	16	16
Baseline	27	30	29	28	26	29	30	28	27	26
High Dose	13	12	12	13	12	16	14	16	13	14
Baseline	30	29	27	28	26	29	28	30	27	26

Depression Data including Baseline



Analysis of covariance: Two factors with interaction and one covariate

$$y_{ijk} = \mu + \alpha_i + \beta_j + \gamma_{ij} + \varphi(x_{ijk} - \bar{x}) + \varepsilon_{ijk}$$

y_{ijk} : Value k in the group with factor level i and j (outcome)

x_{ijk} : Value k in the group with factor level i and j (covariate)

$\bar{x}$: total mean of the x-values

φ : parameter of the covariate x, (regression coefficient within groups)

μ : Total mean

α_i : Deviation group mean i factor 1 vs total mean

β_j : Deviation group mean j factor 2 vs total mean

γ_{ij} : Deviance mean of group ij from $(\mu + \alpha_i + \beta_j)$

ε_{ijk} : Deviation of y_{ijk} from the mean $\mu + \alpha_i + \beta_j$

ε_{ijk} are normally distributed

with mean 0

and fixed variance σ^2

Analysis of covariance: Two factors with interaction and one covariate

$$y_{ijk} = \mu + \alpha_i + \beta_j + \gamma_{ij} + \varphi(x_{ijk} - \bar{X}) + \varepsilon_{ijk}$$

Note:

There are a lot of assumptions contained in this equation.

Especially we assume that the true regression lines in the groups have equal slopes, i.e. they are parallel.

If this assumption is violated, we have to include interaction terms between one or both group factors and the covariate and maybe also between the interaction between group factors and the covariate (3-fold interaction).

It is no problem to analyze these models using available software.

However, interpretation of data becomes very difficult in this case.

Analysis of covariance: Two factors with interaction and one covariate

$$y_{ijk} = \mu + \alpha_i + \beta_j + \gamma_{ij} + \varphi(x_{ijk} - \bar{X}) + \varepsilon_{ijk}$$

We only present the balanced situation. This means we have

- identical Group sizes

- identical average baseline values within groups.

The consequence is that we can attach sums of squares unambiguously to factors, interactions and covariates.

If balancedness is severely violated we recommend to use the method of the GLM (general linear model) which is not presented in this lecture.

The split of the variance

$$\sum_{i=1}^I \sum_{j=1}^J \sum_{k=1}^K \left[y_{ijk} - \bar{y} \right]^2$$

=

$$\sum_{i=1}^I J \bullet K \left(\bar{y}_i - \bar{y} \right)^2 + \sum_{j=1}^J I \bullet K \left(\bar{y}_j - \bar{y} \right)^2 + \sum_{i=1}^I \sum_{j=1}^J K \left(\bar{y}_{ij} - \bar{y}_i - \bar{y}_j + \bar{y} \right)^2$$

$$+ \sum_{i=1}^I \sum_{j=1}^J \sum_{k=1}^K \left(\phi \left(\bar{x}_{ijk} - \bar{x} \right) - \bar{y}_{ij} \right)^2$$

$$+ \sum_{i=1}^I \sum_{j=1}^J \sum_{k=1}^K \left(y_{ijk} - \phi \left(\bar{x}_{ijk} - \bar{x} \right) \right)^2$$

Analysis without Baseline, without interaction

Tests of Between-Subjects Effects

Dependent Variable: depress

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	253,700 ^a	3	84,567	23,145	,000
Intercept	8568,300	1	8568,300	2345,008	,000
geschlecht	,300	1	,300	,082	,777
dosis	253,400	2	126,700	34,676	,000
Error	95,000	26	3,654		
Total	8917,000	30			
Corrected Total	348,700	29			

a. R Squared = ,728 (Adjusted R Squared = ,696)

Analysis without Baseline with interaction

Tests of Between-Subjects Effects

Dependent Variable: depress

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	307,900 ^a	5	61,580	36,224	,000
Intercept	8568,300	1	8568,300	5040,176	,000
geschlecht	,300	1	,300	,176	,678
dosis	253,400	2	126,700	74,529	,000
geschlecht * dosis	54,200	2	27,100	15,941	,000
Error	40,800	24	1,700		
Total	8917,000	30			
Corrected Total	348,700	29			

a. R Squared = ,883 (Adjusted R Squared = ,859)

Analysis with Baseline, without interaction

Tests of Between-Subjects Effects

Dependent Variable: depress

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	275,300 ^a	4	68,825	23,442	,000
Intercept	,001	1	,001	,000	,987
baseline	21,600	1	21,600	7,357	,012
geschlecht	,300	1	,300	,102	,752
dosis	253,400	2	126,700	43,154	,000
Error	73,400	25	2,936		
Total	8917,000	30			
Corrected Total	348,700	29			

a. R Squared = ,790 (Adjusted R Squared = ,756)

Analysis with Baseline, with interaction

Tests of Between-Subjects Effects

Dependent Variable: depress

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	329,500 ^a	6	54,917	65,786	,000
Intercept	,001	1	,001	,001	,976
baseline	21,600	1	21,600	25,875	,000
geschlecht	,300	1	,300	,359	,555
dosis	253,400	2	126,700	151,776	,000
geschlecht * dosis	54,200	2	27,100	32,464	,000
Error	19,200	23	,835		
Total	8917,000	30			
Corrected Total	348,700	29			

a. R Squared = ,945 (Adjusted R Squared = ,931)

Unbalanced Model: Analysis without Baseline, without interaction

Baseline values were modified according to the formula:

Baseline_mod = baseline + dose (1 Placebo, 2 Low, 3 High) + sex (1 male, 2 female)

Tests of Between-Subjects Effects					
Dependent Variable: Depressivität					
Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	253,700 ^a	3	84,567	23,145	,000
Intercept	8568,300	1	8568,300	2345,008	,000
dosis	253,400	2	126,700	34,676	,000
geschl	,300	1	,300	,082	,777
Error	95,000	26	3,654		
Total	8917,000	30			
Corrected Total	348,700	29			
a. R Squared = ,728 (Adjusted R Squared = ,696)					

Unbalanced Model: Analysis with Baseline, without interaction

Tests of Between-Subjects Effects					
Dependent Variable: Depressivität					
Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	275,300 ^a	4	68,825	23,442	,000
Intercept	,241	1	,241	,082	,777
baseline_mod	21,600	1	21,600	7,357	,012
dosis	259,688	2	129,844	44,225	,000
geschl	1,067	1	1,067	,363	,552
Error	73,400	25	2,936		
Total	8917,000	30			
Corrected Total	348,700	29			
a. R Squared = ,790 (Adjusted R Squared = ,756)					

Unbalanced Model: Analysis with interaction, without Baseline

Tests of Between-Subjects Effects					
Dependent Variable: Depressivität					
Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	307,900 ^a	5	61,580	36,224	,000
Intercept	8568,300	1	8568,300	5040,176	,000
dosis	253,400	2	126,700	74,529	,000
geschl	,300	1	,300	,176	,678
dosis * geschl	54,200	2	27,100	15,941	,000
Error	40,800	24	1,700		
Total	8917,000	30			
Corrected Total	348,700	29			
a. R Squared = ,883 (Adjusted R Squared = ,859)					

Unbalanced Model: Analysis with Baseline, with interaction

Tests of Between-Subjects Effects					
Dependent Variable: Depressivität					
Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	329,500 ^a	6	54,917	65,786	,000
Intercept	,241	1	,241	,289	,596
baseline_mod	21,600	1	21,600	25,875	,000
dosis	259,688	2	129,844	155,542	,000
geschl	1,067	1	1,067	1,278	,270
dosis * geschl	54,200	2	27,100	32,464	,000
Error	19,200	23	,835		
Total	8917,000	30			
Corrected Total	348,700	29			
a. R Squared = ,945 (Adjusted R Squared = ,931)					

The Latin Square design

The Latin Square Design

We have already discussed the situation in which per factor combination only one measurement was obtained. Even so this seemed not to be realistic, there are more general situations in which we will use an even more restrictive approach.

Imagine we have three different factors with 5 levels for each factor. Maybe 5 substances are given in 5 concentrations to five different groups of patients. Even if we had only one observation per factor-combination, we would need 125 subjects.

In the following we will see, that it is possible to do this study with only 25 subjects!

The Latin Square Design (Montgomery, page 144)

Factor 2 1	I	II	III	IV	V
1	A	B	C	D	E
2	B	C	D	E	A
3	C	D	E	A	B
4	D	E	A	B	C
5	E	A	B	C	D

Factor 2 1	I	II	III	IV	V
1	24	20	19	24	24
2	17	24	30	27	36
3	18	38	26	27	21
4	26	31	26	23	22
5	22	30	20	29	31

Parameter estimation in the three-factorial latin square design

$$x_{ijk} = \mu + \alpha_i + \beta_j + \gamma_k + \varepsilon_{ijk}$$

$$\hat{\mu} = \bar{x}, \quad \hat{\alpha}_i = \bar{x}_i - \bar{x}, \quad \hat{\beta}_j = \bar{x}_j - \bar{x}, \quad \hat{\gamma}_k = \bar{x}_k - \bar{x}$$

$$x_{ijk} = \bar{x} + (\bar{x}_i - \bar{x}) + (\bar{x}_j - \bar{x}) + (\bar{x}_k - \bar{x}) + \hat{\varepsilon}_{ijk}$$

$$x_{ijk} - (\bar{x} + (\bar{x}_i - \bar{x}) + (\bar{x}_j - \bar{x}) + (\bar{x}_k - \bar{x})) = \hat{\varepsilon}_{ijk}$$

$$x_{ijk} - \bar{x} - \bar{x}_i + \bar{x} - \bar{x}_j + \bar{x} - \bar{x}_k + \bar{x} = \hat{\varepsilon}_{ijk}$$

$$x_{ijk} - \bar{x}_i - \bar{x}_j - \bar{x}_k + 2 \bullet \bar{x} = \hat{\varepsilon}_{ijk}$$

The split of the total sum of squares

Total sum of
squares: SSQ_{total}

Sum of squares
between groups
(Factor 1):
 $SSQ_{\text{between, Factor 1}}$

Sum of squares
between groups
(Factor 2):
 $SSQ_{\text{between, Factor 2}}$

$$\begin{aligned} \sum_{i=1}^I \sum_{j=1}^J \sum_{k=1}^K [x_{ijk} - \bar{x}]^2 &= \sum_{i=1}^I R(\bar{x}_i - \bar{x})^2 + \sum_{j=1}^J R(\bar{x}_j - \bar{x})^2 \\ &+ \sum_{k=1}^K R(\bar{x}_{jk} - \bar{x})^2 + \sum_{i=1}^I \sum_{j=1}^J \sum_{k=1}^K (x_{ijk} - \bar{x}_i - \bar{x}_j - \bar{x}_k + 2 \bullet \bar{x})^2 \end{aligned}$$

Sum of squares
between groups
(Factor 3):
 $SSQ_{\text{between, Factor 3}}$

Sum of squares within groups: SSQ_{within}

Error corrected:

Replaced JK, IK, IJ by R in the SSQ_{between} terms⁶⁷

R is the size of latin square, here $R = 5!$

Three-factorial analysis of variance: tests of significance

$$SSQ_{\text{tot}} = SSQ_{\text{between (Factor 1)}} + SSQ_{\text{between(Faktor 2)}} + SSQ_{\text{between(Faktor 3)}} + SSQ_{\text{within}}$$

$$MSQ_{\text{between (Factor 1)}} = 1 / (I-1) \cdot SSQ_{\text{between (Factor 1)}}$$

$$MSQ_{\text{between (Factor 2)}} = 1 / (J-1) \cdot SSQ_{\text{between (Factor 2)}}$$

$$MSQ_{\text{between (Factor 3)}} = 1 / (K-1) \cdot SSQ_{\text{between (Factor 3)}}$$

$$MSQ_{\text{within}} = 1 / [N-I-J-K+2] \cdot SSQ_{\text{within}}$$

$$F_{\text{factor 1}} = MSQ_{\text{between (Factor 1)}} / MSQ_{\text{within}} \quad DF = I-1, [N-I-J-K+2]$$

$$F_{\text{factor 2}} = MSQ_{\text{between(Factor 2)}} / MSQ_{\text{within}} \quad DF = J-1, [N-I-J-K+2]$$

$$F_{\text{factor 3}} = MSQ_{\text{between(Factor 3)}} / MSQ_{\text{within}} \quad DF = K-1, [N-I-J-K+2]$$

Example: Sum of squares

Sums of squares of the factors

$$SSQ_{\text{Factor 1}} = 68.0 \quad MSQ_{\text{Factor 1}} = 1/4 \bullet 68.0 = 17.0$$

$$SSQ_{\text{Factor 2}} = 150.0 \quad MSQ_{\text{Factor 2}} = 1/4 \bullet 150.0 = 37.5$$

$$SSQ_{\text{Factor 3}} = 330.0 \quad MSQ_{\text{Factor 3}} = 1/4 \bullet 330.0 = 82.5$$

Sums of squares „error“

$$SSQ_{\text{error}} = 128.0 \quad MSQ_{\text{error}} = 1/12 \bullet 128.0 \approx 10.7$$

Example: Test of Significance

Factors

$$F1 = 17.0 / 10.7 \approx 1.594 \Rightarrow p = 0.239$$

$$F2 = 37.5 / 10.7 \approx 3.516 \Rightarrow p = 0.040$$

$$F2 = 82.5 / 10.7 \approx 7.734 \Rightarrow p = 0.003$$

The degrees of freedom are equal to 4,12 for each Test

Example: The anova-tableau

Tests der Zwischensubjekteffekte

Abhängige Variable: value

Quelle	Quadratsumme vom Typ III	df	Mittel der Quadrate	F	Sig.
Korrigiertes Modell	548,000 ^a	12	45,667	4,281	,009
Konstanter Term	16129,000	1	16129,000	1512,094	,000
factor1	68,000	4	17,000	1,594	,239
factor2	150,000	4	37,500	3,516	,040
factor3	330,000	4	82,500	7,734	,003
Fehler	128,000	12	10,667		
Gesamt	16805,000	25			
Korrigierte Gesamtvariation	676,000	24			

a. R-Quadrat = ,811 (korrigiertes R-Quadrat = ,621)

The Graeco-Latin Square design

The Graeco Latin Square Design (Cochran & Cox, page 132)

We can even go one step further and include a fourth factor. But note, that all factors need to have the same number of levels in this approach.

Factor 2 1	I	II	III	IV	V
1	Aa	Bc	Ce	Db	Ed
2	Bb	Cd	Da	Ec	Ae
3	Cc	De	Eb	Ad	Ba
4	Dd	Ea	Ac	Be	Cb
5	Ee	Ab	Bd	Ca	Dc

The Greco Latin Square Design

We can check that every combination of two factors appears exactly once in this design. However, three- and fourfold combinations are not complete.

What are the degrees of freedom?

Total (uncorrected):	$df = 25$
Total (corrected):	$df = 24$
Factor 1 to 4:	$df = 4$
Error:	$df = 25 - 16 - 1 = 8$

And still the split of the Total SSQ is unambiguous for the four factors!

Example: The anova-tableau

Tests der Zwischensubjekteffekte

Abhängige Variable: value

Quelle	Quadratsumme vom Typ III	df	Mittel der Quadrate	F	Sig.
Korrigiertes Modell	610,000 ^a	16	38,125	4,621	,017
Konstanter Term	16129,000	1	16129,000	1955,030	,000
factor1	68,000	4	17,000	2,061	,178
factor2	150,000	4	37,500	4,545	,033
factor3	330,000	4	82,500	10,000	,003
factor4	62,000	4	15,500	1,879	,208
Fehler	66,000	8	8,250		
Gesamt	16805,000	25			
Korrigierte Gesamtvariation	676,000	24			

a. R-Quadrat = ,902 (korrigiertes R-Quadrat = ,707)

The balanced incomplete block design

The balanced incomplete block design (Montgomery, page 154)

Finally we look at a design, where the number of observations is smaller than the possible combinations of two factors:

Factor 2 1	I	II	III	IV
1	73	74		71
2		75	67	72
3	73	75	68	
4	75		72	75

We call this design balanced, even so – obviously – some factor combination appear more frequent (i.e. once) as compared to others (never).

But at least, each factor level appears as often (three times) as each other factor level. If this condition is fulfilled, we don't need necessarily equal number of factor levels for both factors.

The first anova-tableau

Tests der Zwischensubjekteffekte

Abhängige Variable: value

Quelle	Quadratsumme vom Typ I	df	Mittel der Quadrate	F	Sig.
Korrigiertes Modell	77,750 ^a	6	12,958	19,936	,002
Konstanter Term	63075,000	1	63075,000	97038,462	,000
factor1	11,667	3	3,889	5,983	,041
factor2	66,083	3	22,028	33,889	,001
Fehler	3,250	5	,650		
Gesamt	63156,000	12			
Korrigierte Gesamtvariation	81,000	11			

a. R-Quadrat = ,960 (korrigiertes R-Quadrat = ,912)

The second anova-tableau

Tests der Zwischensubjekteffekte

Abhängige Variable: value

Quelle	Quadratsumme vom Typ I	df	Mittel der Quadrate	F	Sig.
Korrigiertes Modell	77,750 ^a	6	12,958	19,936	,002
Konstanter Term	63075,000	1	63075,000	97038,462	,000
factor2	55,000	3	18,333	28,205	,001
factor1	22,750	3	7,583	11,667	,011
Fehler	3,250	5	,650		
Gesamt	63156,000	12			
Korrigierte Gesamtvariation	81,000	11			

a. R-Quadrat = ,960 (korrigiertes R-Quadrat = ,912)

The third anova-tableau

Tests der Zwischensubjekteffekte

Abhängige Variable: value

Quelle	Quadratsumme vom Typ II	df	Mittel der Quadrate	F	Sig.
Korrigiertes Modell	77,750 ^a	6	12,958	19,936	,002
Konstanter Term	63075,000	1	63075,000	97038,462	,000
factor1	22,750	3	7,583	11,667	,011
factor2	66,083	3	22,028	33,889	,001
Fehler	3,250	5	,650		
Gesamt	63156,000	12			
Korrigierte Gesamtvariation	81,000	11			

a. R-Quadrat = ,960 (korrigiertes R-Quadrat = ,912)

Comparison of the three tableaus

Source	TABLEAU		
	I	II	III
Factor 1	11.667	22.750	22.750
Factor 2	66.083	55.000	66.083
Model	77.750	77.750	77.750
Error	3.25	3.25	3.25
Total	81.00	81.00	81.00

In Tableau I and II one of both factors was adjusted for the other. This means it was taken into account that e.g. for factor 1 = 1 there was no measurement with factor 2 = 3. If factor 2 = 3 has larger values than factor 2 = 1, 2, or 4 this has to be corrected, as factor = 1 did not have the chance to „benefit“ from the high values of factor level 3 of factor 2.

Unfortunately we either can correct only one factor with this method (Tableau I and II) or we loose the additivity of SSQ for Factors (Tableau III).