

Stat 424 Final Project

Xiangyu Zeng

Background:

After taking the drugs, blood pressure and blood temperature may vary in response to metabolic demand. An experiment was conducted to determine how this is connected with the type of drugs and the timing to take it. The experiment was conducted by injecting a total of nine rats (subjects) intravenously with three different drugs and in two different time periods in one day. The rats are divided into three groups and each group contains three rats. Different group of rats would be injected by different drugs, and in each group, the rats would be injected once in the morning and once in the night. After each rat had been injected the drug, the blood pressure and blood temperature are measured.

Goal:

The purpose of experiment is to compare the types and the timing and how they make effects on rats' blood pressure and blood temperature.

Attempted Model Determination:

In this section, we make visualization of our data for diagnostic purposes. After analyzing it, we propose that it is a two factor mixed designs (one factor between groups, one factor repeated measures) repeated measures model.

- Boxplots (divided by group for each treatment) with regarded to residuals are made to detect possible relations. We observe there are two outliers in group 3.
- In main effect plot, group 2 and treatment 2 has wider interval than others.
- Interaction plot, we observe no significant interaction.
- Normal Q-Q plot and fitted residual plot (will be used for assumption checking later)

Compute Anova Table and Relevant Statistics

To begin with, we perform analysis of variance (ANOVA) using the **R**function `aoov()` and the results are attached below. Secondly, we compute the means table to obtain various statistics for further hand calculations. The final formal anova table is presented below:

Source	df	SS	MS	F-value	P-value
A	$a - 1 = 2$	$SSA = 100$	$MSA = 50$	9.3168	0.0052
B	$b - 1 = 1$	$SSB = 68.06$	$MSB = 68.06$	12.6812	0.005172
AB	$(a - 1)(b - 1) = 2$	$SSAB = 16.44$	$MSAB = 8.22$	1.5321	0.262778
Subject	$a(s - 1) = 6$	$SSS(A) = 177$	$MSS(A) = 29.5$		
Error	$a(s - 1)(b - 1) = 6$	$SSE = SSB.S(A) = 13$	$MSE = MSB.S(A) = 2.167$		
Total	$abs - 1 = 17$	$SSTotal = 374.5$			

Testing for Interaction Effect (Linear regression, ANOVA)

First, we construct a multivariate model using `lm()`. Then we use ‘`Anova`’ to construct the analysis of the whole model. With the anova output, we first want to test whether interaction difference in means between treatment and subject is statistically significant or not. Test hypothesis are shown below:

$$H_0: \text{all } (\alpha\beta)_{jk} = 0$$

$$H_a: \text{not all } (\alpha\beta)_{jk} \text{ equal zero}$$

From the result of analysis of variance table:

$$F = MSAB/MSB.S(A) = 8.22/2.167 = 3.793$$

According to anova table, we can confirm our previous findings that the main effect of A and B are not significant because the p value is larger than 0.05.

Analysis of Factor Effect: Without Interaction

For level of significance $\alpha = 0.05$, we require $F(.95; 2, 6) = 3.793$. Since $F = 3.793 \leq 5.143$, we conclude null hypothesis, that no significant interaction effects are present. The P-value for the test is 0.262. Based on our previous results that the two factors’ interactions are not significant, we decide to further analyze our data by making multiple pairwise comparison.

Multiple comparison (‘Bonferroni’ & ‘holm’)

(Note: In order to make multiple comparisons, we use reshape library in R to to melt the data to wide format, details can be seen in the R code attached in the appendix.

The multiples for the estimated standard deviation of an estimated contrast of factor A or factor B level means are as follows:

Main A Effect	Main B Effect
Bonferroni procedure	
$B = t[1 - \alpha/2g; \alpha(s - 1)]$	$B = t[1 - \alpha/2g; \alpha(s - 1)(b - 1)]$

There are no specific R functions that could directly compute those values. Hence, by using R as a calculator, we manage to compute p values for the pairwise comparisons (R code are shown in the appendix). The results are all highly significant.

```
[1] 0.002638429 0.015080652 0.041613979
[1] 0.002638429 0.016965734 0.053503687
```

Mauchly's sphericity test

In this section, we want to compare the variances of the differences between all pairs of groups and see whether they are equal. Whereas assumption of normality of distribution may be overlooked from time to time due to the robustness of ANOVA, the assumption of sphericity in repeated measures ANOVA must be met. Violating this may severely compromise the interpretation of the test results. We therefore check by using Mauchly's sphericity test, which is integrated in the function `Anova()` in `car`.

```
Univariate Type III Repeated-Measures ANOVA Assuming Sphericity

              SS num Df Error SS den Df          F      Pr(>F)
(Intercept) 3120.50      1    177          6 105.7797 4.934e-05 ***
G              100.00      2    177          6   1.6949 0.260904
Trials         68.06      1     13          6  31.4103 0.001375 **
G:Trials       16.44      2     13          6   3.7949 0.086064 .
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

As you may see the P-value for 'Group' variable, it returns a p-value 0.26 which is over 0.05 meaning that the null hypothesis, which states that there is no difference in variances for all pairwise group comparisons, is accepted. This means also that we have all rights to go ahead with repeated measure ANOVA (assuming that all other assumptions are met). Note that p-value = 0.086064 for the interaction, which confirms our findings that interaction effect is not significant and there is no need to calculate main effects for A and B.

Model Forecasting

Based on the previous results, our forecasting model (Y) is:

$$Y = 7.833 - 2.667 * G_2 + 1.333 * G_3 + 6.333 * T_2 + 5.167 * S_2 + 6.33 * S_3 - 4.667 * G_2 : T_2 - 2.667 * G_3 : T_2$$

Assumption Checking

- I. In the QQ(quantile-quantile) plot, the plotted points generally fall closely onto the identity line, which suggests that the data come from the normal distribution. So we conclude that the normality assumption is satisfied.
- II. The residuals plots show no violation on constant variance assumption because we did not see "fan" shape or other seasonal patterns there.
- III. From the side-by-side boxplots, it's clear that there are two outliers in the data classified by group and no outliers in the data classified by treatment. We decide retain those outliers in the data since after removing those outliers, the results of our analysis do not change much.

ANCOVA

We could not find any R codes for making analysis of covariance with repeated measures and since it is not advisable to do so, we skip this part of the analysis.

Results / Conclusion

Model (Two factor mixed design - repeated measure):

$$y_{ijk} = \mu + \rho_{i(j)} + \alpha_j + \beta_k + (\alpha\beta)_{jk} + \varepsilon_{ijk}, \quad i=1,2,3, j=1,2,3, k=1,2$$

where y_{ijk} = observation of the i th subject nested in the j th level of factor A, and k th level of factor B. α_j and β_k denote the factor A and factor B main effects, respectively, $(\alpha\beta)_{jk}$ the AB interaction effect. $\rho_{i(j)}$ is the subject (block) random effect, which is nested within factor A. ε_{ij} = errors, independent $N(0, \sigma^2)$.

Note: y_{111} and y_{112} are two observations for the same person/subject, however, y_{112} and y_{122} they are observations for different person/subject, though they have same i .

Assumptions:

$$\rho_{i(j)} \sim_{iid} N(0, \sigma_{\rho}^2), \sum_j \alpha_j = \sum_k \beta_k = \sum_j (\alpha\beta)_{jk} = \sum_k (\alpha\beta)_{jk} = 0, \varepsilon_{ijk} \sim_{iid} N(0, \sigma^2),$$

$\rho_{i(j)}$ and ε_{ijk} are independent.

Base on tests results on interaction effects, we conclude that two factor mixed repeated measure design fits well to our data set. After doing anova test, we also find that the interactions are not significant between groups. Base on this finding, we conduct Bonferroni and holmes multiple comparison, the results of which are highly significant. Then we check different assumptions for our model. In particular, the results from the Mauchly's sphericity test offers strong suggestions that there is no difference in variances for all pairwise group comparisons.

Limitation (how to improve)

- I. Although we find that removing the outliers do not have much significant effects on our results, they still pose certain influences to our analysis results.
- II. Our data set has relatively small size, which makes our model less representative.
- III. Our QQ plot has a light tail, we could have handled it by doing Box Cox transformation to handle the normality issue.

References

Statistical Principles in Experimental Design by Winer(second edition) Table 10.6-6

Applied Linear Statistical Models 5th edition, chapter 27 Repeated Measure Design.

<http://www.itl.nist.gov/div898/handbook/eda/section3/eda336.htm>

<https://gribblelab.wordpress.com/2009/03/09/repeated-measures-anova-using-r/>

<https://www.rdocumentation.org/packages/podkat/versions/1.4.2/topics/p.adjust-methods>