

# Solution to exercise about histamine in dogs, course 02935

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## 1 Introduction

In an experiment with 16 dogs the blood histamine concentration was measured at 0, 1, 3, and 5 minutes after injection of morphine or trimethaphane. Before injection the dogs were classified into two groups according to their level of histamine (intact or depleted).

In the code below we will read in the data and set some of the variables to factors. We will define a treatment variable “TRT” with four levels, combining the two drugs and the two levels of histamin before treatment.

```
histamin <- read.table("histamin.txt", header=T, sep=",", dec=".")
histamin$dog <- factor(histamin$dog)
histamin$minQ <- histamin$min
histamin$min <- as.factor(histamin$min)
histamin$TRT <- as.factor(histamin$treatm:histamin$level)
summary(histamin)
```

```
##      treatm      level      dog      min      hist
## morphine:32  deplet:32   1      : 4   0:16   Min.    :0.020
## trimetha:32  intact:32   2      : 4   1:16   1st Qu.:0.070
##                                     3      : 4   3:16   Median :0.100
##                                     4      : 4   5:16   Mean   :0.312
##                                     5      : 4       3rd Qu.:0.240
##                                     6      : 4       Max.    :3.130
##                                     (Other):40       NA's    :1
##      minQ      TRT
## Min.    :0.00  morphine:deplet:16
## 1st Qu.:0.75  morphine:intact:16
```

```
## Median :2.00   trimetha:deplet:16
## Mean   :2.25   trimetha:intact:16
## 3rd Qu.:3.50
## Max.    :5.00
##
```

In Figure 1 we will plot a line for the level of histamin in each dog during the study.

```
interaction.plot(histamin$minQ,
                 histamin$dog, histamin$hist, xlab="Minute",
                 ylab="Histamin", legend=F,
                 col= histamin$TRT, las=1)
```

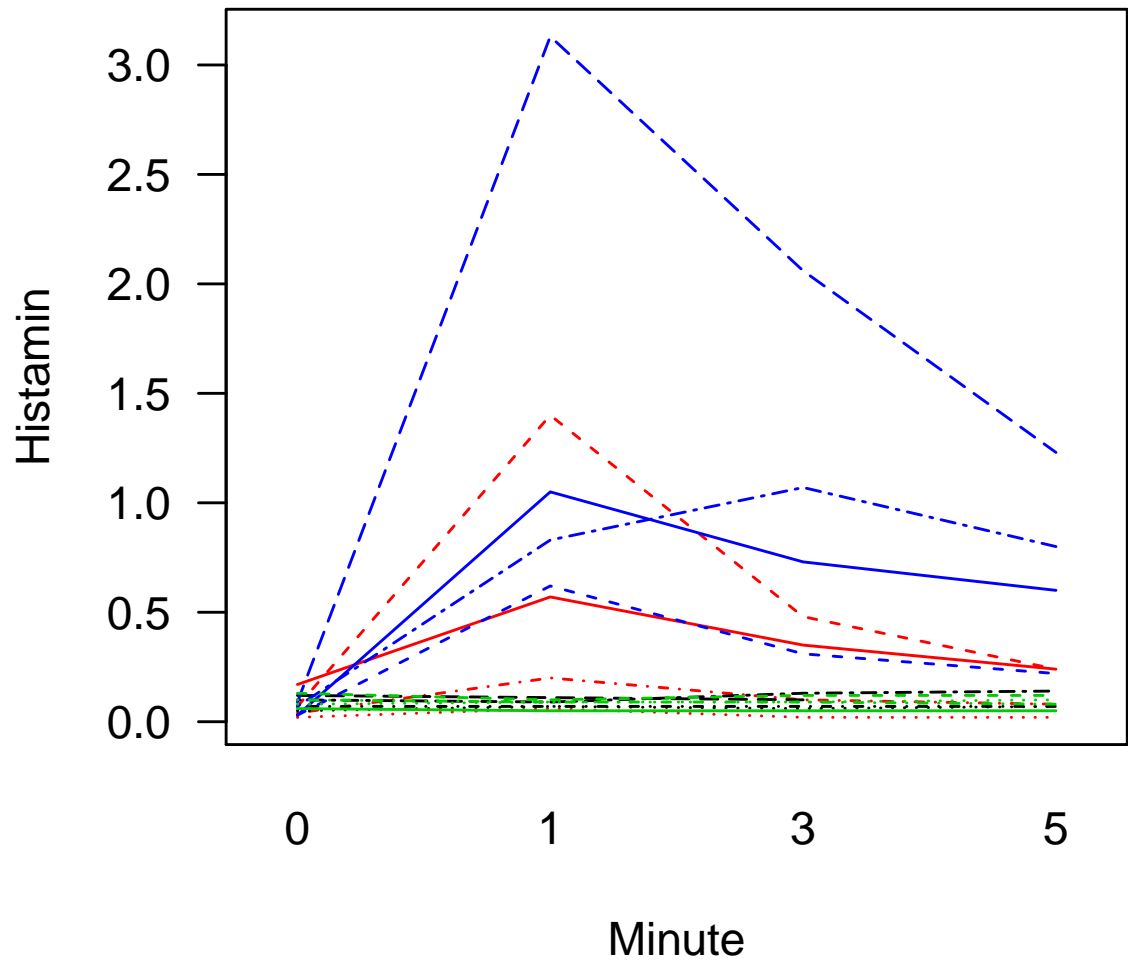
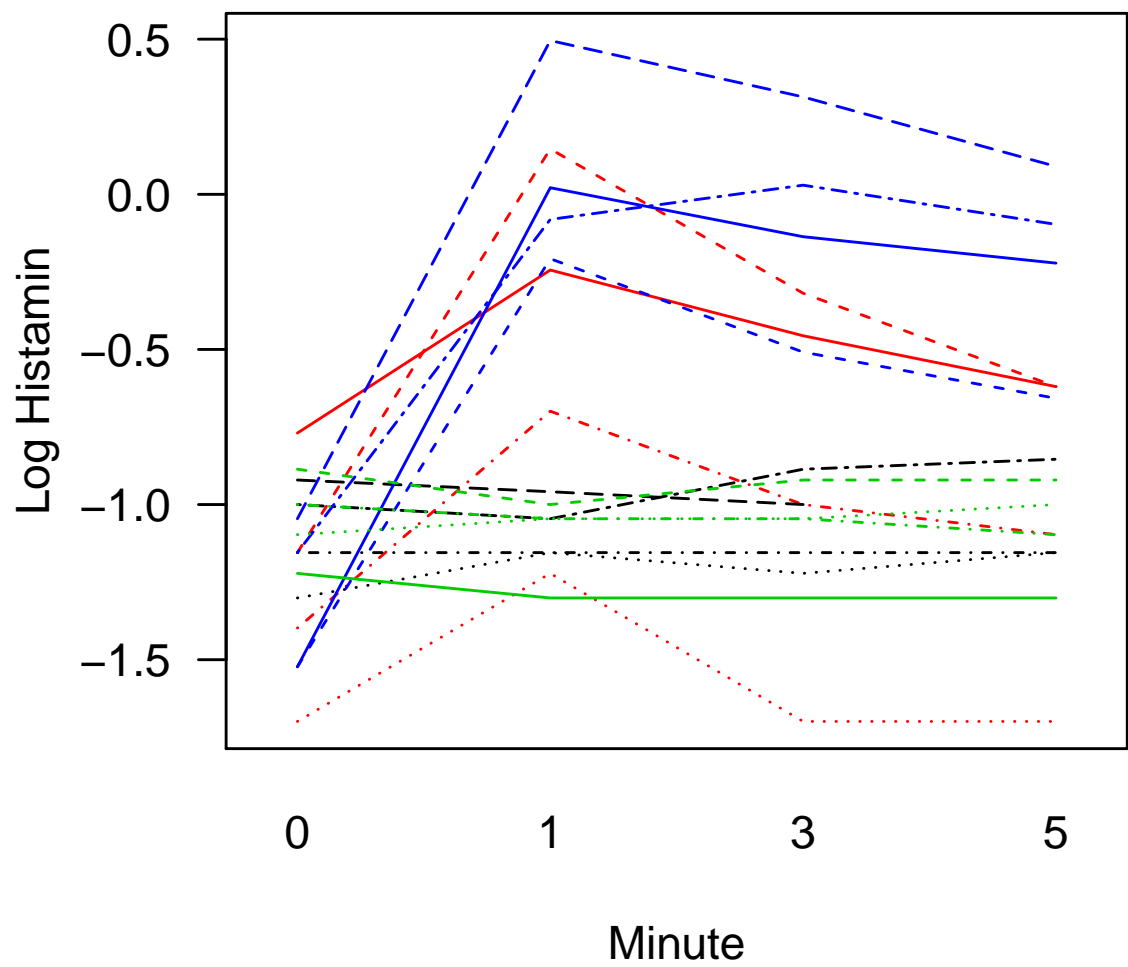


Figure 1: Individual levels of histamin

Looking at Figure 1 we see that the combination trimetha:intact seems to be different. Some of the lines are very close to zero. Perhaps it is easier to look at log transformed data (Figure 2).

```
histamin$loghist <- log10(histamin$hist)
#A line for each dog
interaction.plot(histamin$minQ,
                 histamin$dog, histamin$loghist, xlab="Minute",
                 ylab="Log Histamin", legend=F,
                 col= histamin$TRT, las=1)
```



Figur 2: Individual levels of log-histamin

The patterns in Figure 2 look similar so we could perhaps look at the average in each treatment group (Figure 3).

```
Mean_data <- aggregate(histamin$loghist,
                        by=list(histamin$min, histamin$TRT),
                        mean)
```

*#Have a look*

Mean\_data

```
##      Group.1      Group.2      x
## 1      0 morphine:deplet -1.094188
## 2      1 morphine:deplet -1.078542
## 3      3 morphine:deplet -1.065702
## 4      5 morphine:deplet      NA
## 5      0 morphine:intact -1.255341
## 6      1 morphine:intact -0.504704
## 7      3 morphine:intact -0.868415
## 8      5 morphine:intact -1.008864
## 9      0 trimetha:deplet -1.051204
## 10     1 trimetha:deplet -1.098136
## 11     3 trimetha:deplet -1.078341
## 12     5 trimetha:deplet -1.079690
## 13     0 trimetha:intact -1.311604
## 14     1 trimetha:intact  0.057051
## 15     3 trimetha:intact -0.075516
## 16     5 trimetha:intact -0.221608
```

*#assign names*

```
names(Mean_data) <- c("minute", "TRT", "MeanLogHist")
```

*#Have a look*

Mean\_data

```
##      minute      TRT MeanLogHist
## 1      0 morphine:deplet -1.094188
## 2      1 morphine:deplet -1.078542
## 3      3 morphine:deplet -1.065702
## 4      5 morphine:deplet      NA
## 5      0 morphine:intact -1.255341
## 6      1 morphine:intact -0.504704
## 7      3 morphine:intact -0.868415
## 8      5 morphine:intact -1.008864
## 9      0 trimetha:deplet -1.051204
## 10     1 trimetha:deplet -1.098136
```

```
## 11      3 trimetha:deplet  -1.078341
## 12      5 trimetha:deplet  -1.079690
## 13      0 trimetha:intact   -1.311604
## 14      1 trimetha:intact    0.057051
## 15      3 trimetha:intact   -0.075516
## 16      5 trimetha:intact   -0.221608

#Plot the means

Grp1<-subset(Mean_data,TRT=="morphine:deplet")
Grp2<-subset(Mean_data,TRT=="morphine:intact")
Grp3<-subset(Mean_data,TRT=="trimetha:deplet")
Grp4<-subset(Mean_data,TRT=="trimetha:intact")

plot(as.numeric(Grp1$minute), Grp1$MeanLogHist, type = "b",
     lty=1, xlab = "Minute", las = 1, col= 2,
     ylab = "Mean log histamin", ylim=c(-1.5,1.5))

lines(as.numeric(Grp2$minute), Grp2$MeanLogHist, type = "b", col = 3, lty = 1)
lines(as.numeric(Grp3$minute), Grp3$MeanLogHist, type = "b", col = 4, lty = 1)
lines(as.numeric(Grp4$minute), Grp4$MeanLogHist, type = "b", col = 5, lty = 1)
legend("topleft",
      legend = c("morphine:deplet",
                  "morphine:intact",
                  "trimetha:deplet",
                  "trimetha:intact"),
      title = "Treat",
      lty = c(1,1,1,1),
      col= 2:5)
```

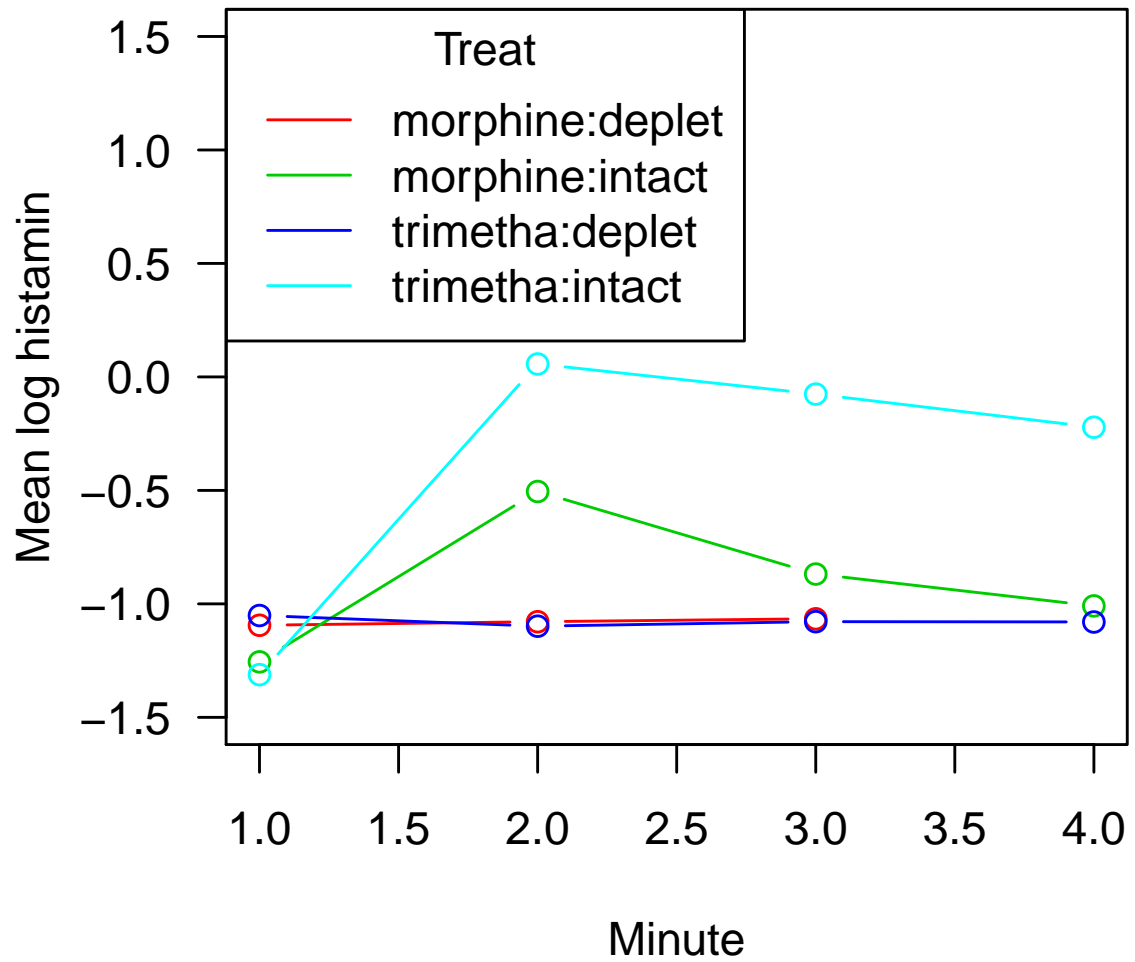


Figure 3: Average log-histamin in treatment groups

From this plot we can see that the histamin levels are unchanged in the groups with depleted histamine levels. For both groups with intact levels of histamine the histamine concentration increases in both groups after injection. There may be a tendency of a higher rise in the trimethaphane group than in the morphine group.



## 2 Analyse these data using one or more of the “simple” methods

As we have seen in the plots we have an example of repeated measurements, as we have four measurements from each dog (one missing however). One way of analyzing these data could be to choose a summary measure representing the treatment effect for each dog. This means that we again just have one observation per dog and we can use our standard methods to analyse the data.

### 2.1 Analysis 1

In the first analysis we could look at the change from 0 to 1 minute (the change from observation 1 to 2).

```
myfun <- function(x){
  x[2]-x[1]
}

Data1 <- aggregate(histamin$loghist,
                    by=list(histamin$dog, histamin$TRT), myfun)

names(Data1) <- c("dog", "TRT", "diff10")

#have a look
Data1
```

##	dog	TRT	diff10
## 1	5	morphine:deplet	-0.045757
## 2	6	morphine:deplet	-0.037789
## 3	7	morphine:deplet	0.000000
## 4	8	morphine:deplet	0.146128
## 5	1	morphine:intact	0.698970
## 6	2	morphine:intact	0.477121
## 7	3	morphine:intact	1.301030
## 8	4	morphine:intact	0.525426
## 9	13	trimetha:deplet	-0.045757
## 10	14	trimetha:deplet	0.051153
## 11	15	trimetha:deplet	-0.113943
## 12	16	trimetha:deplet	-0.079181
## 13	9	trimetha:intact	1.315270
## 14	10	trimetha:intact	1.544068

```
## 15 11 trimetha:intact 1.073980
## 16 12 trimetha:intact 1.541302

# Analyse the change from 0 to 1
reg1 <- lm(diff10 ~ TRT, data = Data1)

#Model check
par(mfrow=c(2,2))
plot(reg1, which=1:4)
par(mfrow=c(1,1))
```

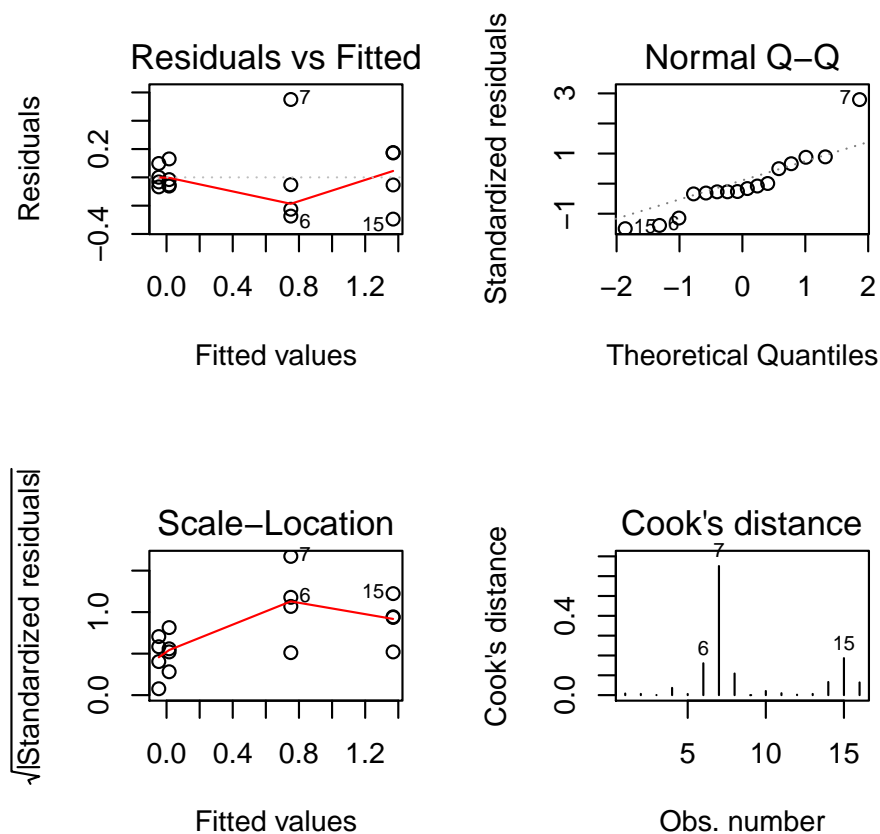


Figure 4: Plots for checking simple model 1

In this analysis we are using the change from observation 1 to 2 as our outcome. This results in a one-way analysis of variance.

The model check could perhaps have been better (mainly due to observation 7) but we only have 16 observations so it is difficult to be too sure. The results of the analysis are seen below.

```
summary(reg1)

##
## Call:
## lm(formula = diff10 ~ TRT, data = Data1)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -0.2947 -0.0628 -0.0420  0.1062  0.5504
##
## Coefficients:
##              Estimate Std. Error t value Pr(>|t|)
## (Intercept)      0.0156     0.1137   0.14  0.89283
## TRTmorphine:intact  0.7350     0.1608   4.57  0.00064 ***
## TRTtrimetha:deplet -0.0626     0.1608  -0.39  0.70394
## TRTtrimetha:intact  1.3530     0.1608   8.42  2.2e-06 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.227 on 12 degrees of freedom
## Multiple R-squared:  0.897, Adjusted R-squared:  0.871
## F-statistic: 34.8 on 3 and 12 DF, p-value: 3.37e-06

confint(reg1)

##              2.5 %   97.5 %
## (Intercept)   -0.23207 0.26336
## TRTmorphine:intact  0.38467 1.08531
## TRTtrimetha:deplet -0.41290 0.28774
## TRTtrimetha:intact  1.00269 1.70333

anova(reg1)

## Analysis of Variance Table
##
## Response: diff10
##      Df Sum Sq Mean Sq F value   Pr(>F)
## TRT     3    5.40    1.799    34.8 3.4e-06 ***
## Residuals 12    0.62    0.052
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

From this analysis (the anova table) we see a significant treatment effect ( $p < 0.0001$ ). The two groups with intact levels have significantly higher increases compared to the reference group (morphine:deplet). Perhaps we would also like to compare the two intact groups directly. We can follow our one-way analysis up by pairwise testing.

```
pairwise.t.test(Data1$diff10, Data1$TRT, p.adj = "none")

##
## Pairwise comparisons using t tests with pooled SD
##
## data: Data1$diff10 and Data1$TRT
##
##               morphine:deplet morphine:intact trimetha:deplet
## morphine:intact 0.00064          -              -
## trimetha:deplet 0.70394          0.00033          -
## trimetha:intact 2.2e-06          0.00234          1.4e-06
##
## P value adjustment method: none
```

Looking at the pairwise tests we can see also a significant difference between trimetha:intact and morphine:intact  $p = 0.00234$

## 2.2 Analysis 2

Another possibility for a summary statistic could be to look at the maximum value.

```
Data2 <- aggregate(histamin$loghist,
                    by=list(histamin$dog, histamin$TRT), max)

names(Data2) <- c("dog", "TRT", "max")

#have a look
Data2

##      dog      TRT      max
## 1     5 morphine:deplet -0.853872
## 2     6 morphine:deplet      NA
## 3     7 morphine:deplet -1.154902
## 4     8 morphine:deplet -1.154902
## 5     1 morphine:intact -0.698970
## 6     2 morphine:intact -1.221849
```

```
## 7    3 morphine:intact  0.146128
## 8    4 morphine:intact -0.244125
## 9   13 trimetha:deplet -1.000000
## 10  14 trimetha:deplet -1.000000
## 11  15 trimetha:deplet -0.886057
## 12  16 trimetha:deplet -1.221849
## 13    9 trimetha:intact -0.207608
## 14  10 trimetha:intact  0.021189
## 15  11 trimetha:intact  0.029384
## 16  12 trimetha:intact  0.495544

# Analyse the max
reg2 <- lm(max ~ TRT, data = Data2)

#Model check
par(mfrow=c(2,2))
plot(reg2, which=1:4)
par(mfrow=c(1,1))
```

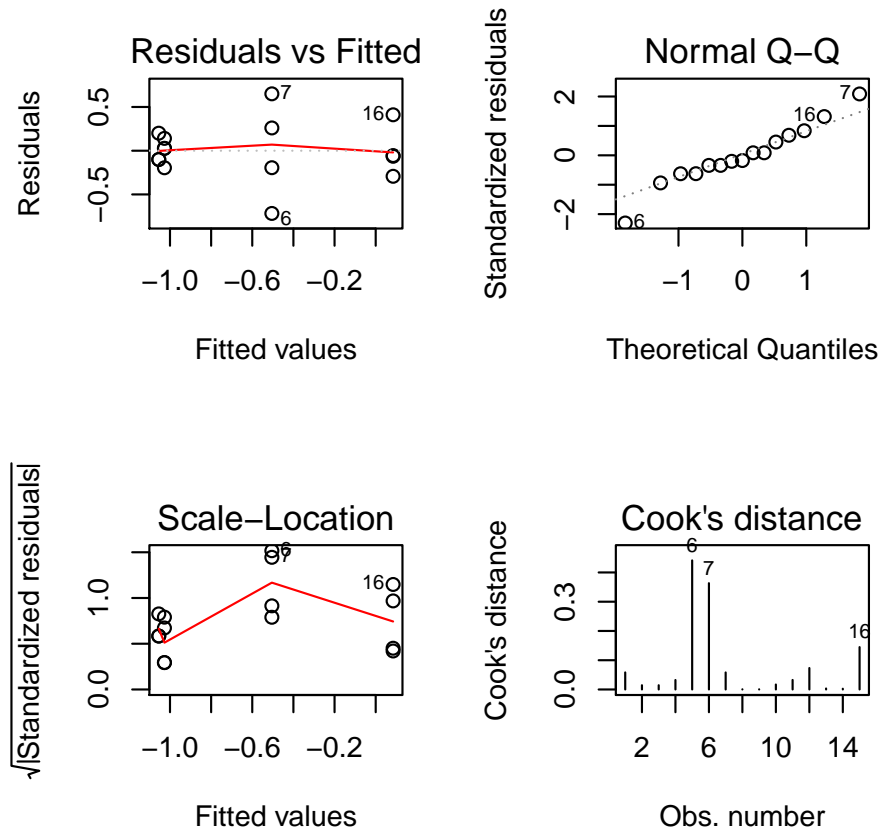


Figure 5: Plots for checking simple model 2

In the following we have the results of the analysis looking at the maximum value for each dog.

```
summary(reg2)

##
## Call:
## lm(formula = max ~ TRT, data = Data2)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -0.7171 -0.1473 -0.0552  0.1708  0.6508
##
## Coefficients:
```

```
##               Estimate Std. Error t value Pr(>|t|)
## (Intercept)      -1.0546     0.2078   -5.08  0.00036 ***
## TRTmorphine:intact  0.5499     0.2749    2.00  0.07074 .
## TRTtrimetha:deplet  0.0276     0.2749    0.10  0.92187
## TRTtrimetha:intact  1.1392     0.2749    4.14  0.00163 **
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.36 on 11 degrees of freedom
## (1 observation deleted due to missingness)
## Multiple R-squared:  0.696, Adjusted R-squared:  0.613
## F-statistic: 8.39 on 3 and 11 DF,  p-value: 0.00349

confint(reg2)

##               2.5 %    97.5 %
## (Intercept)      -1.511857 -0.59726
## TRTmorphine:intact -0.055094  1.15480
## TRTtrimetha:deplet -0.577366  0.63253
## TRTtrimetha:intact  0.534238  1.74413

anova(reg2)

## Analysis of Variance Table
##
## Response: max
##          Df Sum Sq Mean Sq F value Pr(>F)
## TRT         3   3.26    1.09    8.39 0.0035 **
## Residuals  11   1.42    0.13
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

From this analysis (the anova table) we see a significant treatment effect ( $p = 0.0035$ ). The trimetha:intact group has a significantly higher maximum compared to the reference group (morphine:deplet). Again we can follow our one-way analysis up by pairwise testing.

```
pairwise.t.test(Data2$max, Data2$TRT, p.adj = "none")

##
## Pairwise comparisons using t tests with pooled SD
##
## data:  Data2$max and Data2$TRT
```

```
##
##           morphine:deplet morphine:intact trimetha:deplet
## morphine:intact 0.0707      -              -
## trimetha:deplet 0.9219      0.0647        -
## trimetha:intact 0.0016      0.0409        0.0011
##
## P value adjustment method: none
```

Looking at the pairwise tests we can see also a significant difference between trimetha:intact and morphine:intact  $p=0.0409$

## 2.3 Analysis 3

We could also look at the change from 0 to 5 minutes (observations 1 and 4), the change from baseline.

```
myfun2 <- function(x){
  x[4]-x[1]
}

Data3 <- aggregate(histamin$loghist,
  by=list(histamin$dog, histamin$TRT), myfun2)

names(Data3) <- c("dog", "TRT", "diff50")

#have a look
Data3

##      dog      TRT      diff50
## 1      5 morphine:deplet 0.146128
## 2      6 morphine:deplet      NA
## 3      7 morphine:deplet 0.000000
## 4      8 morphine:deplet 0.146128
## 5      1 morphine:intact 0.301030
## 6      2 morphine:intact 0.000000
## 7      3 morphine:intact 0.535113
## 8      4 morphine:intact 0.149762
## 9     13 trimetha:deplet -0.096910
## 10     14 trimetha:deplet 0.096910
## 11     15 trimetha:deplet -0.034762
## 12     16 trimetha:deplet -0.079181
## 13      9 trimetha:intact 0.865301
## 14     10 trimetha:intact 1.301030
```



```
## 15 11 trimetha:intact 1.057992
## 16 12 trimetha:intact 1.135663

# Analyse the change from 0 to 5
reg3 <- lm(diff50 ~ TRT, data = Data3)

#Model check
par(mfrow=c(2,2))
plot(reg3, which=1:4)
par(mfrow=c(1,1))
```

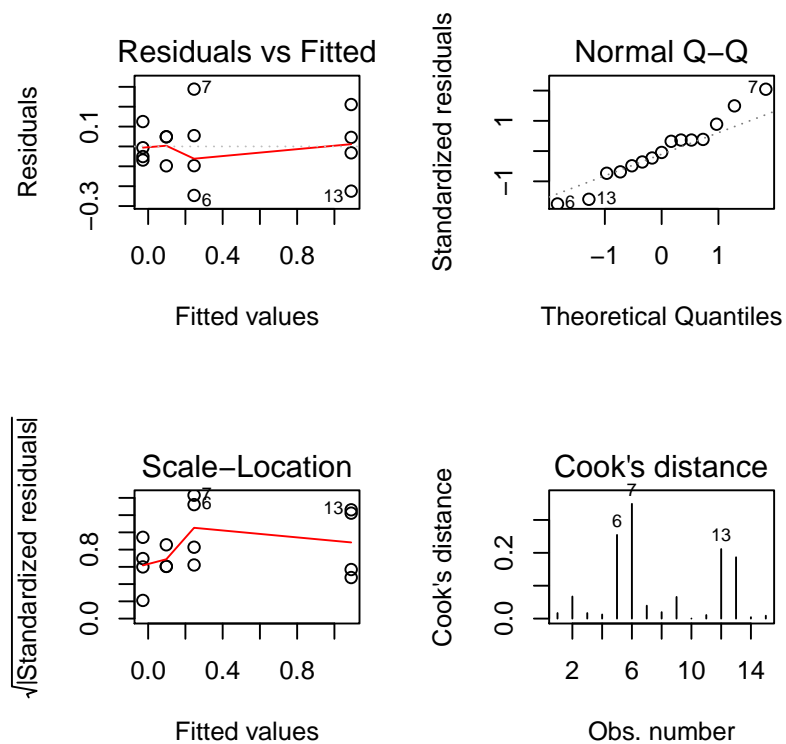


Figure 6: Plots for checking initial model

In the following we have the results of the analysis looking at the change from baseline for each dog.

```
summary(reg3)

##
## Call:
## lm(formula = diff50 ~ TRT, data = Data3)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -0.24648 -0.08257 -0.00628  0.05163  0.28864
##
## Coefficients:
##              Estimate Std. Error t value Pr(>|t|)
## (Intercept)      0.0974     0.0940   1.04    0.32
## TRTmorphine:intact  0.1491     0.1244   1.20    0.26
## TRTtrimetha:deplet -0.1259     0.1244  -1.01    0.33
## TRTtrimetha:intact  0.9926     0.1244   7.98 6.7e-06 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.163 on 11 degrees of freedom
## (1 observation deleted due to missingness)
## Multiple R-squared:  0.911, Adjusted R-squared:  0.887
## F-statistic: 37.6 on 3 and 11 DF, p-value: 4.48e-06

confint(reg3)

##              2.5 %   97.5 %
## (Intercept)   -0.10954 0.30438
## TRTmorphine:intact -0.12472 0.42284
## TRTtrimetha:deplet -0.39969 0.14788
## TRTtrimetha:intact  0.71880 1.26636

anova(reg3)

## Analysis of Variance Table
##
## Response: diff50
##      Df Sum Sq Mean Sq F value Pr(>F)
## TRT    3  2.993   0.998   37.6 4.5e-06 ***
## Residuals 11  0.292   0.027
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

From this analysis (the anova table) we see a significant treatment effect ( $p < 0.0001$ ). The trimetha:intact group has a significantly higher change from baseline compared to the reference group (morphine:deplet). Again we can follow our one-way analysis up by pairwise testing.

```
pairwise.t.test(Data3$diff50, Data3$TRT, p.adj = "none")

##
## Pairwise comparisons using t tests with pooled SD
##
## data: Data3$diff50 and Data3$TRT
##
##               morphine:deplet morphine:intact trimetha:deplet
## morphine:intact 0.256          -                -
## trimetha:deplet 0.333          0.036             -
## trimetha:intact 6.7e-06        1.5e-05           9.9e-07
##
## P value adjustment method: none
```

Looking at the pairwise tests we can see that it is mainly trimetha:intact group, which is different from the other three. There is also a significant difference between trimetha:intact and morphine:intact  $p < 0.0001$ .

All in all we get more or less the same conclusions which ever analysis we have chosen. We see that the two depleted groups are very similar and that the trimetha:intact group changes most and significantly more than the morphine:intact group. It depends on the summary measure whether we can see differences between morphine:intact and the depleted groups.

### 3 Using a Random Effects Model

Instead of using the summary measures we could also apply a random effects model making use of all the observations at once. Below we have a simple random effects model with a random effect of dog and an interaction between minute and treatment allowing the differences between the treatment groups to vary at each minute (Often the groups will be close at the beginning).

```
library(nlme)
model1 <- lme(loghist ~ min + TRT + min:TRT,
              random = ~1 | dog, data = histamin, na.action = na.omit)

anova(model1)
```

```
##          numDF denDF F-value p-value
## (Intercept)      1    35 121.793 <.0001
## min              3    35  59.857 <.0001
## TRT              3    12   4.315 0.0278
## min:TRT          9    35  28.724 <.0001
```

From the results we can see that there is a significant interaction between min:TRT ( $p < .0001$ ). To find out where the differences lie one can look at a table of the fixed effects estimates.

```
summary(model1)$tTable
```

```
##          Value Std.Error DF  t-value    p-value
## (Intercept) -1.094188  0.163753 35 -6.68196 9.8693e-08
## min1         0.015645  0.082070 35  0.19064 8.4991e-01
## min3         0.028486  0.082070 35  0.34709 7.3060e-01
## min5         0.077696  0.090638 35  0.85721 3.9716e-01
## TRTmorphine:intact -0.161153 0.231581 12 -0.69588 4.9976e-01
## TRTtrimetha:deplet  0.042984 0.231581 12  0.18561 8.5585e-01
## TRTtrimetha:intact -0.217417 0.231581 12 -0.93884 3.6633e-01
## min1:TRTmorphine:intact 0.734991 0.116065 35  6.33259 2.8225e-07
## min3:TRTmorphine:intact 0.358440 0.116065 35  3.08827 3.9273e-03
## min5:TRTmorphine:intact 0.168780 0.122273 35  1.38035 1.7623e-01
## min1:TRTtrimetha:deplet -0.062578 0.116065 35 -0.53916 5.9319e-01
## min3:TRTtrimetha:deplet -0.055623 0.116065 35 -0.47924 6.3475e-01
## min5:TRTtrimetha:deplet -0.106182 0.122273 35 -0.86840 3.9109e-01
## min1:TRTtrimetha:intact 1.353010 0.116065 35 11.65735 1.3246e-13
## min3:TRTtrimetha:intact 1.207602 0.116065 35 10.40454 2.9731e-12
## min5:TRTtrimetha:intact 1.012300 0.122273 35  8.27900 9.2733e-10
```

The intercept is the expected log-histamine level for a dog in morphine:deplet at minute 0. At minute 1 the expected log-histamine for trimetha:intact would be :  $-1.094188 + 0.015645 - 0.217417 + 1.353010 = 0.05705$ .

We also had a random effect and the estimated variances are seen below.

```
#The estimates random effects
```

```
VarCorr(model1)
```

```
## dog = pdLogChol(1)
##          Variance StdDev
## (Intercept) 0.093789 0.30625
## Residual    0.013471 0.11606
```

```
#intra class correlation  
0.09378856/(0.09378856+0.01347106)  
  
## [1] 0.87441
```

The intraclass correlation describes how strongly measurements from the same dog resemble each other. Here we have a quite high value and we could also see in Figure 2 that even though the dogs have similar patterns then some dogs generally have high levels and some low.

### 3.1 Model check

As for the linear model we still have assumptions underlying the model that should be checked. First we will check the assumptions concerning the residual error  $\epsilon_i \sim N(0, \sigma^2)$ . This is like what we did for the linear models but we have to construct more of the residual plots ourselves.

```
#residual plot  
plot(model1)  
  
#qqplot for residuals  
qqnorm(residuals(model1))  
qqline(residuals(model1))
```

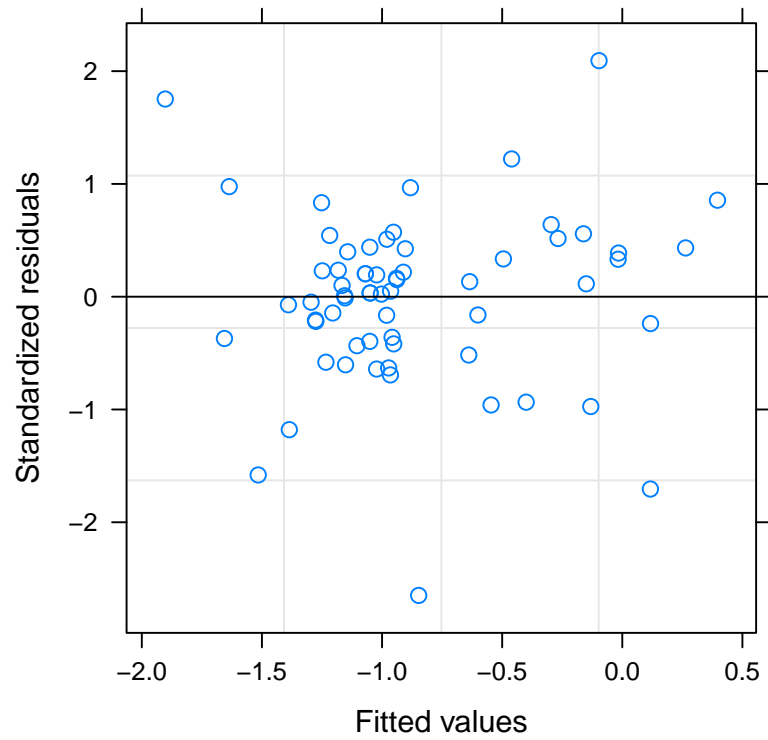
From Figure 7 we see in the top plot that it looks like a random scatter of dots, so the variance homogeneity looks ok and the qq plot looks like a straight line so the normality of residuals is ok.

In Figure 8 we are checking the normal distribution of the random effect.

```
#qqplot for random effect  
qqnorm(ranef(model1)$"(Intercept)")  
qqline(ranef(model1)$"(Intercept)")
```

This approach will only make sense if the number of levels for a factor is not too small (here we have 16 dogs).

In this example the assumptions underlying the model seem ok.



### Normal Q-Q Plot

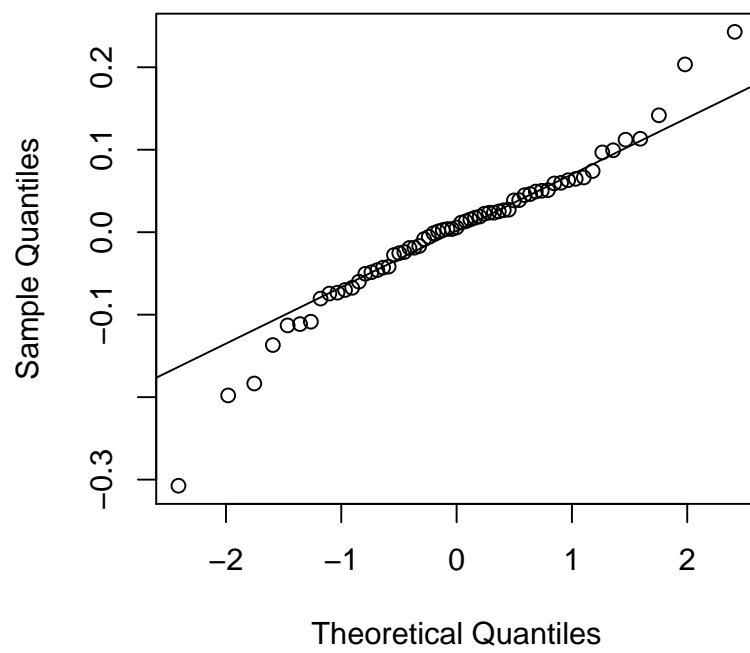
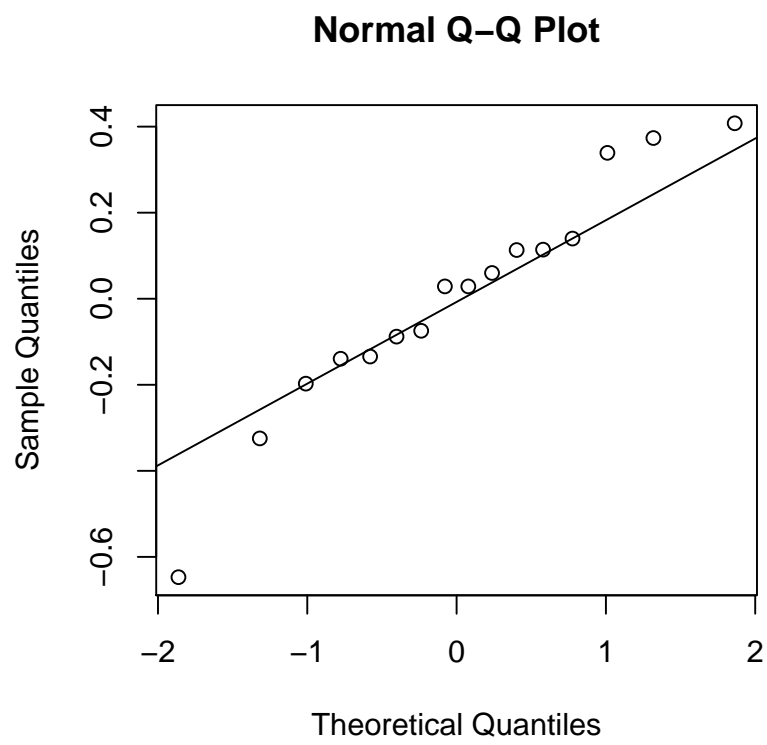


Figure 7: Residual plots for checking residual error



Figur 8: QQ plot for random effect