Final Project – Second Draft

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Contents

Questions/next steps

- Questions for Kan:
  - Samples from different organs?
  - Differences among isotypes?
- Why are there 7 treatment groups now?
  - Treatment 1-3: different doses for 1st drug
  - Treatment 4-6: different doses for 2nd drug
  - Treatment 7: control
- Instead of looking at average of Binding, look at average of reactivity to see the percentage of reactive
- Variances can be very different in different cells

Data Preparation

```
## Response [https://raw.githubusercontent.com/luokan1227/537P1/master/Data.xlsx]
##   Date: 2020-10-24 16:54
##   Status: 200
##   Content-Type: application/octet-stream
##   Size: 341 kB
## <ON DISK>  C:\Users\shihn\AppData\Local\Temp\RtmpGYqh4X\file3ae424133f39.xlsx

## Response [https://raw.githubusercontent.com/luokan1227/537P1/master/MonkeyID.xlsx]
##   Date: 2020-10-24 16:54
```

```
##   Status: 200
##   Content-Type: application/octet-stream
##   Size: 50.1 kB
## <ON DISK>  C:\Users\shihn\AppData\Local\Temp\RtmpGYqh4X\file3ae4649f2484.xlsx
```

Introduction

Methodologies

Data Summaries

Contingency Tables

The study included 20 rhesus macaques.

```
table(Data2$MonkeyID)
```

```
##
## 6104 6105 6107 6117 6118 6119 6125 6132 6160 6193 6199 6200 6201 6202 6203 6204
##   35  228  239  243    7   55  216  251  183  117   48  191   73   78  238  156
## 6205 6209 6210 6214
##    5   46   50    6
```

There are four time points; one before any procedure was done, and three after vaccine shots were administered to the macaques. In the treatment groups, groups 1-3 represent different doses of drug 1, groups 4-6 represent different doses of drug 2, and group 7 represents the control group. Later we'll look at the effect made by different drugs first and then different doses.

```
table(Data2$Time_Point, Data2$Treatment)
```

```
##
##     group 1 group 2 group 3 group 4 group 5 group 6 group 7
##   0     129       0       0      90       0       0      54
##   1     190      60      96     105     297     131     125
##   2     141     110       0     148      77       0     347
##   3     122       0       0     101       0       0     142
```

```
table(Data2$Drug, Data2$Treatment)
```

```
##
##     group 1 group 2 group 3 group 4 group 5 group 6 group 7
##   1     582     170      96       0       0       0       0
##   2       0       0       0     444     374     131       0
##   3       0       0       0       0       0       0     668
```

Now we look at the table of each marcaque and the corresponding treatment group. As the table shows, each marcaque only received one kidn of treatment.

```
table(Data2$MonkeyID, Data2$Treatment)
```

```
##
##          group 1 group 2 group 3 group 4 group 5 group 6 group 7
##   6104         0       0      35       0       0       0       0
##   6105         0       0       0     228       0       0       0
##   6107         0       0       0       0       0       0     239
##   6117       243       0       0       0       0       0       0
##   6118         0       7       0       0       0       0       0
```

```
##   6119       0        0       55       0       0       0       0
##   6125       0        0        0     216       0       0       0
##   6132       0        0        0       0     251       0       0
##   6160     183        0        0       0       0       0       0
##   6193       0      117        0       0       0       0       0
##   6199       0        0        0       0       0      48       0
##   6200       0        0        0       0       0       0     191
##   6201       0        0        0       0      73       0       0
##   6202       0        0        0       0       0      78       0
##   6203       0        0        0       0       0       0     238
##   6204     156        0        0       0       0       0       0
##   6205       0        0        0       0       0       5       0
##   6209       0       46        0       0       0       0       0
##   6210       0        0        0       0      50       0       0
##   6214       0        0        6       0       0       0       0
```

```r
ggplot(Data2, aes(x = Drug, y = Binding)) + geom_boxplot(aes(group = Drug))
```
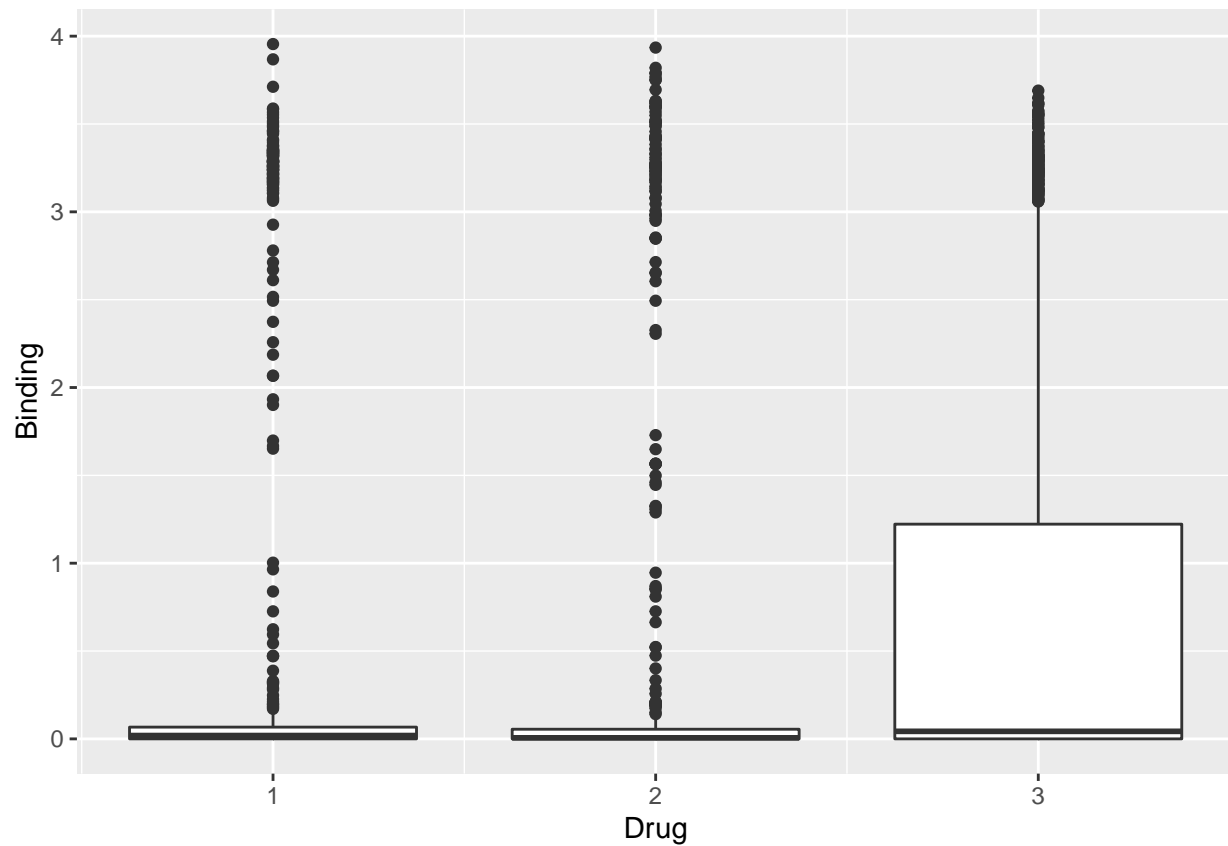


```r
table(Data$Drug, Data$Reactivity)
```

```
##
##       0    1
##   1 680  168
##   2 807  142
##   3 464  204
```

```r
table(Data$Time_Point, Data$Reactivity)
```

```
## 
##       0   1
##   0 249  24
##   1 971  33
##   2 475 348
##   3 256 109
```

```
Data2 %>% group_by(Drug) %>% summarize(avgLMuFreq = mean(LMuFreq), avgHMuFreq = mean(HMuFreq), avgBindi
```
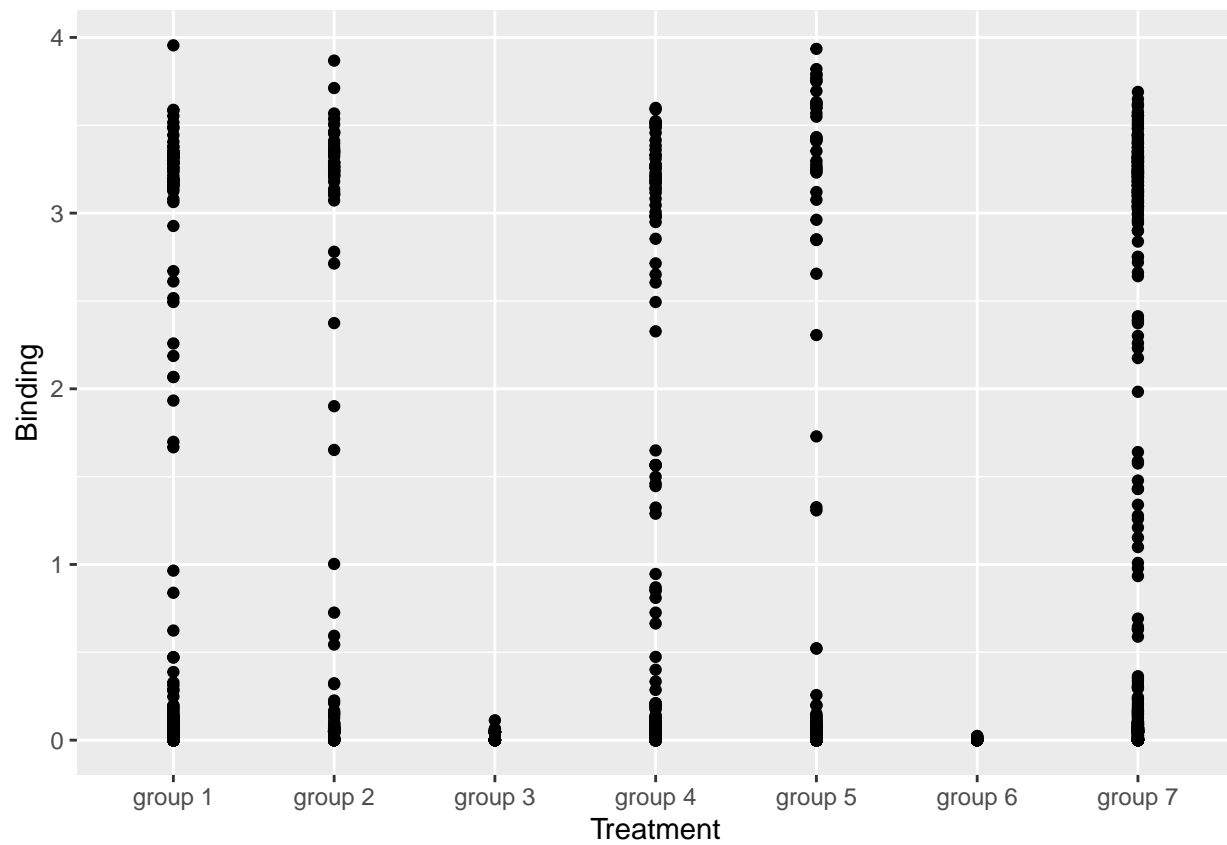
```
## `summarise()` ungrouping output (override with `.groups` argument)
```

```
## # A tibble: 3 x 6
##    Drug avgLMuFreq avgHMuFreq avgBinding varBinding avgReact
##   <dbl>      <dbl>      <dbl>      <dbl>      <dbl>    <dbl>
## 1     1     0.0616        NA       0.450       1.14    0.198
## 2     2     0.0616    0.0730       0.334       0.864   0.150
## 3     3     0.0594    0.0559       0.807       1.81    0.305
```
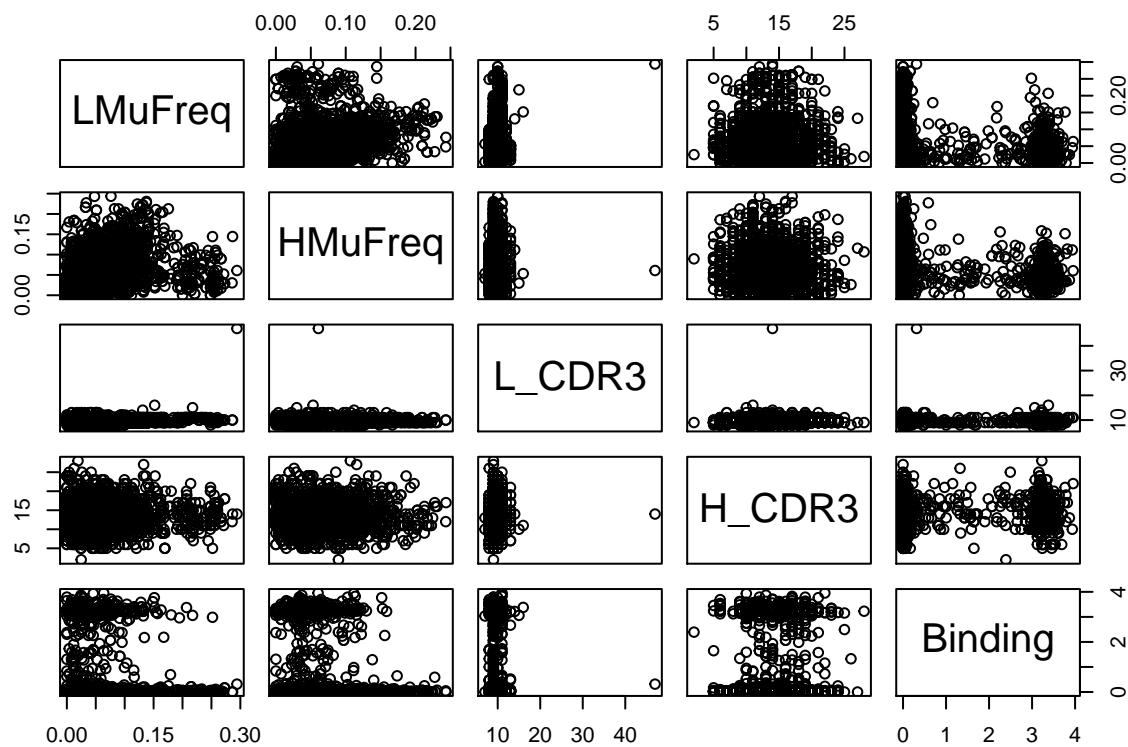
```
ggplot(Data2, aes(x = Drug, y = Binding)) + geom_point()
```
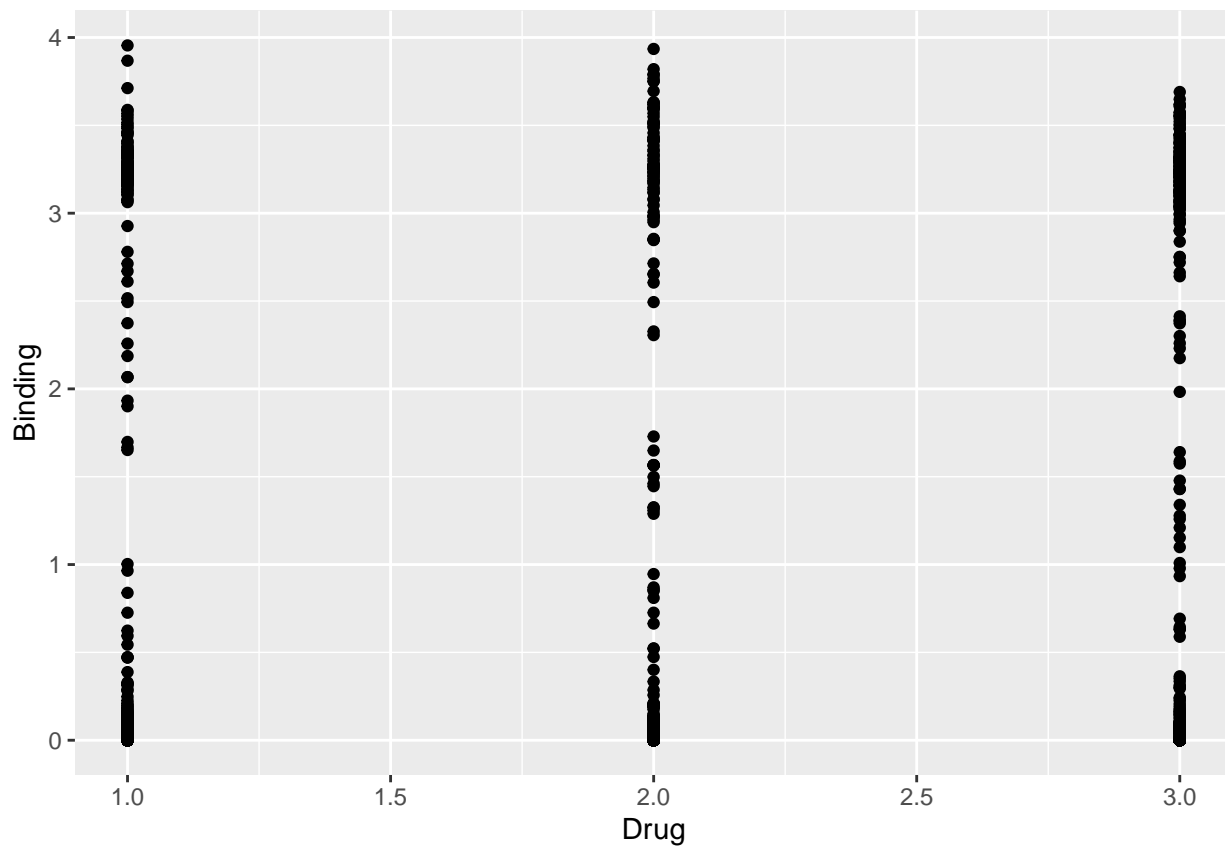


```
ggplot(Data2, aes(x = Treatment, y = Binding)) + geom_point()
```

```r
Data2 %>% select(LMuFreq, HMuFreq, L_CDR3, H_CDR3, Binding) %>% pairs()
```

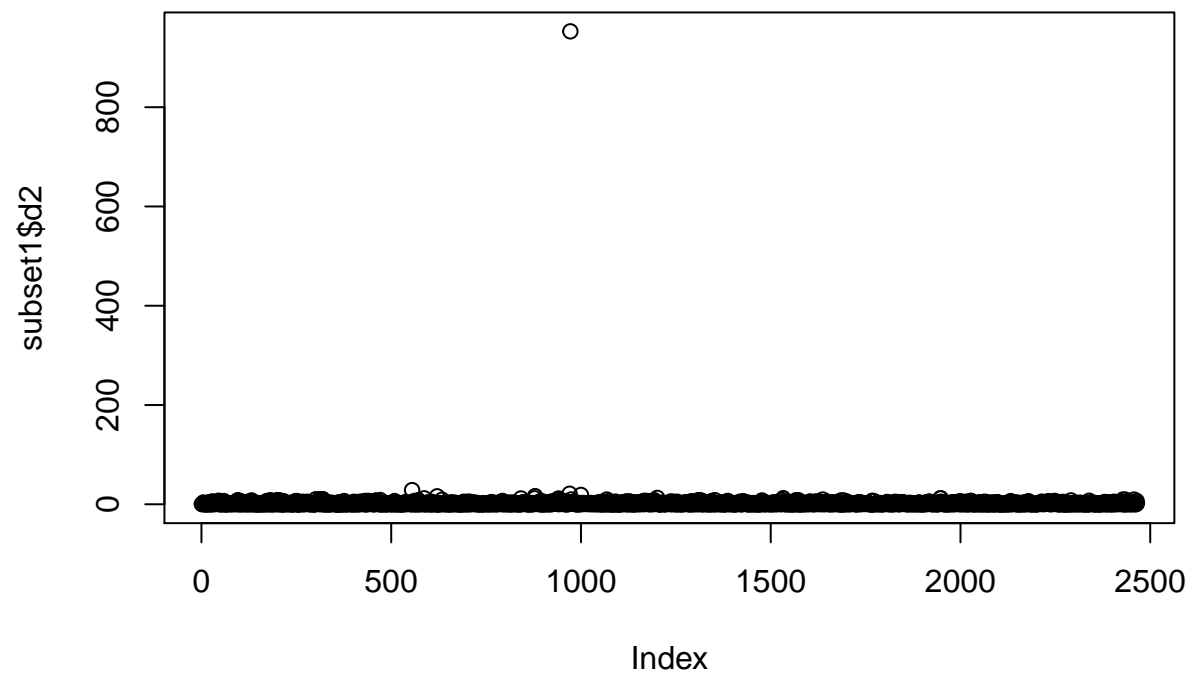## Outlier detection

Notice may have outlier in LCDR3 variable.
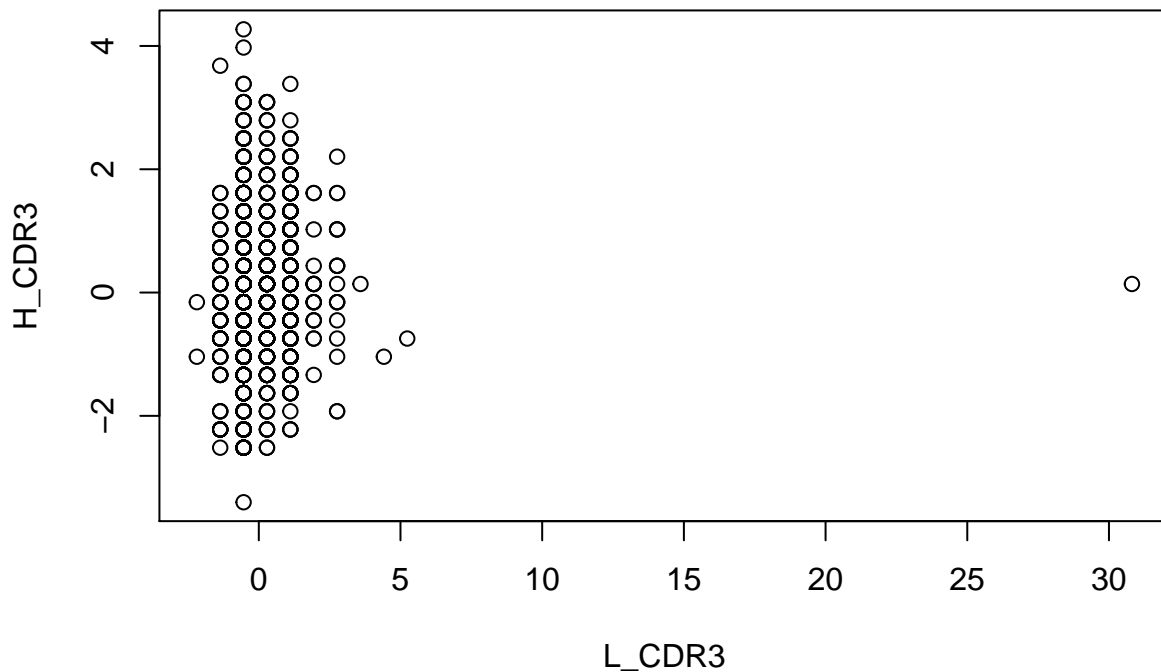
```r
summary(Data2$L_CDR3)
```

```
##    Min. 1st Qu.  Median    Mean 3rd Qu.    Max.
##    7.00    9.00    9.00    9.65   10.00   47.00
```

```r
subset1 <- Data2 %>% select(L_CDR3, H_CDR3)
subset1$d2 <- mahalanobis(subset1, colMeans(subset1), cov(subset1))
subset1$Z <- scale(subset1)
plot(subset1$d2)
```

```
plot(subset1$Z)
```

```r
subset2 <- subset1 %>% arrange(desc(d2), desc(Z))
subset2[1,]
```

```
## # A tibble: 1 x 4
##   L_CDR3 H_CDR3    d2 Z[,"L_CDR3"] [,"H_CDR3"] [,"d2"]
##    <dbl>  <dbl> <dbl>        <dbl>       <dbl>   <dbl>
## 1     47     14  953.         30.8       0.139    49.4
```

```r
which(subset1$L_CDR3 == 47)
```

```
## [1] 972
```

Row 972 from `Data2` is in fact an outlier, as shown in the summary and plots above. The value for `L_CDR3` is quite unlikely. Since we can't go back to the original data, we remove the data point and will use the new dataset `Data3`.

```r
Data2[972,]
```

```
## # A tibble: 1 x 24
##   MonkeyID  Drug Treatment Time_Point Isotype HV_Extract HD_Extract HJ_Extract
##      <dbl> <dbl> <chr>          <dbl> <chr>   <chr>      <chr>      <chr>
## 1     6107     3 group 7            2 G       3          2          4
## # ... with 16 more variables: H_VBase <dbl>, H_Substitutions <dbl>,
## #   H_Insertions <dbl>, H_Deletions <dbl>, HMuFreq <dbl>, H_CDR3 <dbl>,
## #   LV_Extract <chr>, LJ_Extract <chr>, L_VBase <dbl>, L_Substitutions <dbl>,
## #   L_Insertions <dbl>, L_Deletions <dbl>, LMuFreq <dbl>, L_CDR3 <dbl>,
## #   Binding <dbl>, Reactivity <dbl>
```

```
Data3 <- Data2[-972,]
```

Multivariate Data Analysis

```
ID <- as.factor(Data3$MonkeyID)
trt <- as.factor(Data3$Treatment)
drug <- as.factor(Data3$Drug)
tp <- as.factor(Data3$Time_Point)
it <- as.factor(Data3$Isotype)
# four-way manova


fit.manova <- manova(cbind(Data3$L_CDR3, Data3$LMuFreq, Data3$H_CDR3, Data3$HMuFreq, Data3$Binding) ~ d:
summary(fit.manova)

##              Df  Pillai approx F num Df den Df    Pr(>F)
## drug          2 0.06176   15.626     10   4904 < 2.2e-16 ***
## tp            3 0.19800   34.667     15   7359 < 2.2e-16 ***
## Residuals  2455
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
fit.gls <- lm(cbind(Data3$L_CDR3, Data3$LMuFreq, Data3$H_CDR3, Data3$HMuFreq, Data3$Binding) ~ drug + tp
summary(fit.gls)

## Response Data3$L_CDR3 :
##
## Call:
## lm(formula = `Data3$L_CDR3` ~ drug + tp)
##
## Residuals:
##     Min      1Q  Median      3Q     Max
## -2.5951 -0.6553 -0.5665  0.4049  6.2942
##
## Coefficients:
##             Estimate Std. Error t value Pr(>|t|)
## (Intercept)  9.65526    0.06178 156.281   <2e-16 ***
## drug2       -0.04384    0.04543  -0.965    0.335
## drug3       -0.11063    0.05064  -2.184    0.029 *
## tp1          0.01725    0.06568   0.263    0.793
## tp2          0.05051    0.06748   0.749    0.454
## tp3          0.02183    0.07682   0.284    0.776
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.9508 on 2455 degrees of freedom
##   (3 observations deleted due to missingness)
## Multiple R-squared:  0.002056,   Adjusted R-squared:  2.318e-05
## F-statistic: 1.011 on 5 and 2455 DF,  p-value: 0.4092
##
##
## Response Data3$LMuFreq :
##
```

```
## Call:
## lm(formula = `Data3$LMuFreq` ~ drug + tp)
##
## Residuals:
##       Min       1Q   Median       3Q      Max
## -0.07233 -0.03662 -0.01504  0.01987  0.22320
##
## Coefficients:
##               Estimate Std. Error t value Pr(>|t|)
## (Intercept)  0.0719614  0.0035016   20.551  < 2e-16 ***
## drug2       -0.0001371  0.0025748   -0.053  0.95752
## drug3        0.0003640  0.0028703    0.127  0.89910
## tp1         -0.0084782  0.0037224   -2.278  0.02284 *
## tp2         -0.0147355  0.0038247   -3.853  0.00012 ***
## tp3         -0.0189684  0.0043541   -4.356 1.38e-05 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.05389 on 2455 degrees of freedom
##   (3 observations deleted due to missingness)
## Multiple R-squared:  0.01006,    Adjusted R-squared:  0.008045
## F-statistic:  4.99 on 5 and 2455 DF,  p-value: 0.0001496
##
##
## Response Data3$H_CDR3 :
##
## Call:
## lm(formula = `Data3$H_CDR3` ~ drug + tp)
##
## Residuals:
##       Min       1Q   Median       3Q      Max
## -11.2921  -2.1012  -0.1012   1.8988  14.7079
##
## Coefficients:
##             Estimate Std. Error t value Pr(>|t|)
## (Intercept) 13.95536    0.21869  63.813  < 2e-16 ***
## drug2       -0.40087    0.16080  -2.493  0.01273 *
## drug3       -0.57162    0.17926  -3.189  0.00145 **
## tp1         -0.45325    0.23248  -1.950  0.05134 .
## tp2         -0.09164    0.23887  -0.384  0.70128
## tp3          0.69302    0.27193   2.549  0.01088 *
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 3.366 on 2455 degrees of freedom
##   (3 observations deleted due to missingness)
## Multiple R-squared:  0.01637,    Adjusted R-squared:  0.01436
## F-statistic:  8.17 on 5 and 2455 DF,  p-value: 1.162e-07
##
##
## Response Data3$HMuFreq :
##
## Call:
## lm(formula = `Data3$HMuFreq` ~ drug + tp)
```

```
## 
## Residuals:
##       Min        1Q    Median        3Q       Max
## -0.085072 -0.029604 -0.006174  0.024701  0.174404
## 
## Coefficients:
##              Estimate Std. Error t value Pr(>|t|)
## (Intercept)  0.079295   0.002713  29.230  < 2e-16 ***
## drug2        0.005776   0.001995   2.896 0.003816 **
## drug3       -0.008182   0.002224  -3.679 0.000239 ***
## tp1         -0.010457   0.002884  -3.626 0.000294 ***
## tp2         -0.016569   0.002963  -5.592 2.50e-08 ***
## tp3         -0.021684   0.003373  -6.428 1.55e-10 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
## 
## Residual standard error: 0.04175 on 2455 degrees of freedom
##   (3 observations deleted due to missingness)
## Multiple R-squared:  0.04463,    Adjusted R-squared:  0.04268
## F-statistic: 22.94 on 5 and 2455 DF,  p-value: < 2.2e-16
## 
## 
## Response Data3$Binding :
## 
## Call:
## lm(formula = `Data3$Binding` ~ drug + tp)
## 
## Residuals:
##     Min      1Q  Median      3Q     Max
## -1.1488 -0.8238 -0.0266  0.0164  3.2987
## 
## Coefficients:
##              Estimate Std. Error t value Pr(>|t|)
## (Intercept)  0.028715   0.065234   0.440    0.660
## drug2       -0.028075   0.047967  -0.585    0.558
## drug3        0.074364   0.053473   1.391    0.164
## tp1          0.009551   0.069347   0.138    0.890
## tp2          1.045709   0.071253  14.676   <2e-16 ***
## tp3          0.751695   0.081115   9.267   <2e-16 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
## 
## Residual standard error: 1.004 on 2455 degrees of freedom
##   (3 observations deleted due to missingness)
## Multiple R-squared:  0.1975, Adjusted R-squared:  0.1958
## F-statistic: 120.8 on 5 and 2455 DF,  p-value: < 2.2e-16
```

```r
fit.logit <- lm(Data3$Reactivity ~ drug*tp)
summary(fit.logit)
```

```
## 
## Call:
## lm(formula = Data3$Reactivity ~ drug * tp)
## 
## Residuals:
```

```
##       Min      1Q   Median       3Q      Max
## -0.45954 -0.30282 -0.04624 -0.00800  0.99200
##
## Coefficients:
##               Estimate Std. Error t value Pr(>|t|)
## (Intercept)  0.108527   0.032132   3.378 0.000743 ***
## drug2        0.002584   0.050123   0.052 0.958890
## drug3       -0.108527   0.059152  -1.835 0.066668 .
## tp1         -0.062284   0.037649  -1.654 0.098182 .
## tp2          0.333704   0.039536   8.440  < 2e-16 ***
## tp3          0.112784   0.046089   2.447 0.014471 *
## drug2:tp1   -0.018808   0.056100  -0.335 0.737458
## drug3:tp1    0.070284   0.070352   0.999 0.317874
## drug2:tp2   -0.102593   0.060290  -1.702 0.088949 .
## drug3:tp2    0.125834   0.066442   1.894 0.058357 .
## drug2:tp3    0.162243   0.070163   2.312 0.020839 *
## drug3:tp3    0.190033   0.074355   2.556 0.010655 *
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.365 on 2452 degrees of freedom
## Multiple R-squared:  0.196,  Adjusted R-squared:  0.1924
## F-statistic: 54.34 on 11 and 2452 DF,  p-value: < 2.2e-16
```

Pairwise comparison

Now we take a look at the pairwise comparison for each treatment group.

```r
respMat <- as.matrix(Data3[,c("L_CDR3", "LMuFreq", "H_CDR3", "HMuFreq", "Binding")])
# pairwise comparison among treatment groups
fit1 <- manova(respMat[,1:5] ~ trt)
# summary(fit1)

vars <- c("L_CDR3", "LMuFreq", "H_CDR3", "HMuFreq", "Binding")

p <- 5
q1 <- length(unique(trt))
alpha.old <- 0.05
nc1 <- p*q1*(q1-1)/2
alpha.new1 <- alpha.old/nc1

for (i in 1:5){
  w <- c(0, 0, 0, 0, 0)
  w[i] <- 1
  print(paste(vars[i], " pairwise CI's"))
  cont <- contrast(emmeans(fit1, "trt", weights = w), "pairwise")
  bb <- confint(cont, level = 1 - alpha.new1, adj = "none")
  print(bb)
}
```

```
## [1] "L_CDR3  pairwise CI's"

## Note: Use 'contrast(regrid(object), ...)' to obtain contrasts of back-transformed estimates

##  contrast          estimate      SE   df lower.CL upper.CL
##  group 1 - group 2  0.08842 0.0829 2454  -0.2016    0.378
```

```
## group 1 - group 3 -0.03487 0.1047 2454   -0.4012    0.331
## group 1 - group 4  0.11097 0.0599 2454   -0.0987    0.321
## group 1 - group 5 -0.01158 0.0630 2454   -0.2321    0.209
## group 1 - group 6  0.08361 0.0919 2454   -0.2380    0.405
## group 1 - group 7  0.11559 0.0540 2454   -0.0732    0.304
## group 2 - group 3 -0.12328 0.1213 2454   -0.5477    0.301
## group 2 - group 4  0.02255 0.0857 2454   -0.2773    0.322
## group 2 - group 5 -0.10000 0.0879 2454   -0.4075    0.208
## group 2 - group 6 -0.00480 0.1105 2454   -0.3913    0.382
## group 2 - group 7  0.02717 0.0816 2454   -0.2584    0.313
## group 3 - group 4  0.14583 0.1070 2454   -0.2283    0.520
## group 3 - group 5  0.02328 0.1087 2454   -0.3571    0.404
## group 3 - group 6  0.11848 0.1277 2454   -0.3282    0.565
## group 3 - group 7  0.15046 0.1037 2454   -0.2124    0.513
## group 4 - group 5 -0.12255 0.0667 2454   -0.3559    0.111
## group 4 - group 6 -0.02735 0.0945 2454   -0.3579    0.303
## group 4 - group 7  0.00462 0.0582 2454   -0.1990    0.208
## group 5 - group 6  0.09520 0.0965 2454   -0.2423    0.433
## group 5 - group 7  0.12717 0.0614 2454   -0.0876    0.342
## group 6 - group 7  0.03198 0.0908 2454   -0.2857    0.350
##
## Results are averaged over the levels of: rep.meas
## Note: contrasts are still on the [.: scale
## Confidence level used: 0.999523809523809
## [1] "LMuFreq  pairwise CI's"

## Note: Use 'contrast(regrid(object), ...)' to obtain contrasts of back-transformed estimates

##  contrast            estimate      SE   df lower.CL upper.CL
## group 1 - group 2  0.007328 0.00472 2454 -0.00918  0.02384
## group 1 - group 3 -0.006001 0.00596 2454 -0.02686  0.01486
## group 1 - group 4  0.002551 0.00341 2454 -0.00939  0.01449
## group 1 - group 5 -0.000993 0.00359 2454 -0.01355  0.01156
## group 1 - group 6 -0.001342 0.00523 2454 -0.01966  0.01697
## group 1 - group 7  0.003112 0.00307 2454 -0.00764  0.01386
## group 2 - group 3 -0.013330 0.00691 2454 -0.03750  0.01084
## group 2 - group 4 -0.004778 0.00488 2454 -0.02185  0.01230
## group 2 - group 5 -0.008322 0.00500 2454 -0.02583  0.00919
## group 2 - group 6 -0.008670 0.00629 2454 -0.03068  0.01334
## group 2 - group 7 -0.004216 0.00465 2454 -0.02048  0.01205
## group 3 - group 4  0.008552 0.00609 2454 -0.01275  0.02986
## group 3 - group 5  0.005008 0.00619 2454 -0.01665  0.02667
## group 3 - group 6  0.004659 0.00727 2454 -0.02077  0.03009
## group 3 - group 7  0.009114 0.00591 2454 -0.01155  0.02978
## group 4 - group 5 -0.003544 0.00380 2454 -0.01683  0.00974
## group 4 - group 6 -0.003893 0.00538 2454 -0.02271  0.01493
## group 4 - group 7  0.000562 0.00331 2454 -0.01103  0.01216
## group 5 - group 6 -0.000349 0.00549 2454 -0.01957  0.01887
## group 5 - group 7  0.004106 0.00350 2454 -0.00812  0.01633
## group 6 - group 7  0.004454 0.00517 2454 -0.01364  0.02254
##
## Results are averaged over the levels of: rep.meas
## Note: contrasts are still on the [.: scale
## Confidence level used: 0.999523809523809
## [1] "H_CDR3  pairwise CI's"
```

```
## Note: Use 'contrast(regrid(object), ...)' to obtain contrasts of back-transformed estimates

##   contrast          estimate    SE   df lower.CL upper.CL
##   group 1 - group 2   0.5757 0.295 2454 -0.45488    1.606
##   group 1 - group 3   0.9285 0.372 2454 -0.37339    2.230
##   group 1 - group 4   0.9693 0.213 2454  0.22406    1.715
##   group 1 - group 5   0.3928 0.224 2454 -0.39095    1.177
##   group 1 - group 6   0.7509 0.327 2454 -0.39208    1.894
##   group 1 - group 7   0.6651 0.192 2454 -0.00594    1.336
##   group 2 - group 3   0.3528 0.431 2454 -1.15543    1.861
##   group 2 - group 4   0.3936 0.305 2454 -0.67188    1.459
##   group 2 - group 5  -0.1829 0.312 2454 -1.27567    0.910
##   group 2 - group 6   0.1753 0.393 2454 -1.19820    1.549
##   group 2 - group 7   0.0894 0.290 2454 -0.92557    1.104
##   group 3 - group 4   0.0408 0.380 2454 -1.28891    1.371
##   group 3 - group 5  -0.5357 0.386 2454 -1.88738    0.816
##   group 3 - group 6  -0.1776 0.454 2454 -1.76477    1.410
##   group 3 - group 7  -0.2634 0.369 2454 -1.55299    1.026
##   group 4 - group 5  -0.5765 0.237 2454 -1.40570    0.253
##   group 4 - group 6  -0.2184 0.336 2454 -1.39301    0.956
##   group 4 - group 7  -0.3042 0.207 2454 -1.02780    0.419
##   group 5 - group 6   0.3581 0.343 2454 -0.84126    1.558
##   group 5 - group 7   0.2723 0.218 2454 -0.49084    1.035
##   group 6 - group 7  -0.0858 0.323 2454 -1.21483    1.043
##
## Results are averaged over the levels of: rep.meas
## Note: contrasts are still on the [.: scale
## Confidence level used: 0.999523809523809
## [1] "HMuFreq  pairwise CI's"

## Note: Use 'contrast(regrid(object), ...)' to obtain contrasts of back-transformed estimates

##   contrast          estimate      SE   df lower.CL  upper.CL
##   group 1 - group 2  0.001782 0.00367 2454 -0.01105  0.014613
##   group 1 - group 3  0.014768 0.00463 2454 -0.00144  0.030977
##   group 1 - group 4 -0.000904 0.00265 2454 -0.01018  0.008375
##   group 1 - group 5 -0.005563 0.00279 2454 -0.01532  0.004195
##   group 1 - group 6 -0.009779 0.00407 2454 -0.02401  0.004452
##   group 1 - group 7  0.013055 0.00239 2454  0.00470  0.021410
##   group 2 - group 3  0.012986 0.00537 2454 -0.00579  0.031765
##   group 2 - group 4 -0.002686 0.00379 2454 -0.01595  0.010580
##   group 2 - group 5 -0.007345 0.00389 2454 -0.02095  0.006261
##   group 2 - group 6 -0.011561 0.00489 2454 -0.02866  0.005539
##   group 2 - group 7  0.011273 0.00361 2454 -0.00136  0.023910
##   group 3 - group 4 -0.015672 0.00473 2454 -0.03223  0.000884
##   group 3 - group 5 -0.020331 0.00481 2454 -0.03716 -0.003502
##   group 3 - group 6 -0.024547 0.00565 2454 -0.04431 -0.004785
##   group 3 - group 7 -0.001713 0.00459 2454 -0.01777  0.014343
##   group 4 - group 5 -0.004659 0.00295 2454 -0.01498  0.005665
##   group 4 - group 6 -0.008875 0.00418 2454 -0.02350  0.005750
##   group 4 - group 7  0.013959 0.00258 2454  0.00495  0.022968
##   group 5 - group 6 -0.004216 0.00427 2454 -0.01915  0.010717
##   group 5 - group 7  0.018618 0.00272 2454  0.00912  0.028120
##   group 6 - group 7  0.022834 0.00402 2454  0.00878  0.036891
##
```

```
## Results are averaged over the levels of: rep.meas
## Note: contrasts are still on the [.: scale
## Confidence level used: 0.999523809523809
## [1] "Binding  pairwise CI's"

## Note: Use 'contrast(regrid(object), ...)' to obtain contrasts of back-transformed estimates

##  contrast          estimate     SE   df lower.CL upper.CL
##  group 1 - group 2  -0.5394 0.0951 2454  -0.8721  -0.2066
##  group 1 - group 3   0.3560 0.1202 2454  -0.0644   0.7763
##  group 1 - group 4  -0.0201 0.0688 2454  -0.2608   0.2205
##  group 1 - group 5   0.0164 0.0723 2454  -0.2366   0.2695
##  group 1 - group 6   0.3819 0.1055 2454   0.0129   0.7510
##  group 1 - group 7  -0.4236 0.0619 2454  -0.6403  -0.2070
##  group 2 - group 3   0.8953 0.1392 2454   0.4083   1.3823
##  group 2 - group 4   0.5192 0.0983 2454   0.1752   0.8633
##  group 2 - group 5   0.5558 0.1009 2454   0.2029   0.9086
##  group 2 - group 6   0.9213 0.1268 2454   0.4778   1.3648
##  group 2 - group 7   0.1157 0.0937 2454  -0.2120   0.4434
##  group 3 - group 4  -0.3761 0.1227 2454  -0.8055   0.0532
##  group 3 - group 5  -0.3395 0.1248 2454  -0.7760   0.0969
##  group 3 - group 6   0.0259 0.1465 2454  -0.4866   0.5384
##  group 3 - group 7  -0.7796 0.1190 2454  -1.1960  -0.3632
##  group 4 - group 5   0.0366 0.0765 2454  -0.2311   0.3043
##  group 4 - group 6   0.4021 0.1084 2454   0.0228   0.7813
##  group 4 - group 7  -0.4035 0.0668 2454  -0.6371  -0.1699
##  group 5 - group 6   0.3655 0.1107 2454  -0.0218   0.7528
##  group 5 - group 7  -0.4401 0.0704 2454  -0.6865  -0.1937
##  group 6 - group 7  -0.8056 0.1042 2454  -1.1701  -0.4410
##
## Results are averaged over the levels of: rep.meas
## Note: contrasts are still on the [.: scale
## Confidence level used: 0.999523809523809
```

```r
# pairwise comparison among drug groups
fit2 <- manova(respMat[,1:5] ~ drug)
# summary(fit2)
p <- 5
q2 <- length(unique(drug))
alpha.old <- 0.05
nc2 <- p*q2*(q2-1)/2
alpha.new2 <- alpha.old/nc2

for (i in 1:5){
  w <- c(0, 0, 0, 0, 0)
  w[i] <- 1
  print(paste(vars[i], " pairwise CI's"))
  cont <- contrast(emmeans(fit2, "drug", weights = w), "pairwise")
  bb <- confint(cont, level = 1 - alpha.new2, adj = "none")
  print(bb)
}
```

```
## [1] "L_CDR3  pairwise CI's"

## Note: Use 'contrast(regrid(object), ...)' to obtain contrasts of back-transformed estimates

##  contrast estimate     SE   df lower.CL upper.CL
```

```
##  1 - 2       0.0451 0.0450 2458  -0.0870    0.177
##  1 - 3       0.1018 0.0492 2458  -0.0429    0.246
##  2 - 3       0.0567 0.0480 2458  -0.0844    0.198
##
## Results are averaged over the levels of: rep.meas
## Note: contrasts are still on the [.: scale
## Confidence level used: 0.996666666666667
## [1] "LMuFreq  pairwise CI's"

## Note: Use 'contrast(regrid(object), ...)' to obtain contrasts of back-transformed estimates

##  contrast  estimate      SE   df lower.CL upper.CL
##  1 - 2    -0.000176 0.00256 2458 -0.00770  0.00735
##  1 - 3     0.002320 0.00280 2458 -0.00592  0.01056
##  2 - 3     0.002496 0.00273 2458 -0.00554  0.01053
##
## Results are averaged over the levels of: rep.meas
## Note: contrasts are still on the [.: scale
## Confidence level used: 0.996666666666667
## [1] "H_CDR3  pairwise CI's"

## Note: Use 'contrast(regrid(object), ...)' to obtain contrasts of back-transformed estimates

##  contrast estimate    SE   df lower.CL upper.CL
##  1 - 2      0.4907 0.160 2458   0.0204    0.961
##  1 - 3      0.4438 0.175 2458  -0.0712    0.959
##  2 - 3     -0.0469 0.171 2458  -0.5492    0.456
##
## Results are averaged over the levels of: rep.meas
## Note: contrasts are still on the [.: scale
## Confidence level used: 0.996666666666667
## [1] "HMuFreq  pairwise CI's"

## Note: Use 'contrast(regrid(object), ...)' to obtain contrasts of back-transformed estimates

##  contrast estimate      SE   df lower.CL  upper.CL
##  1 - 2     -0.006 0.00199 2458 -0.01186 -0.000145
##  1 - 3      0.011 0.00218 2458  0.00461  0.017432
##  2 - 3      0.017 0.00213 2458  0.01076  0.023276
##
## Results are averaged over the levels of: rep.meas
## Note: contrasts are still on the [.: scale
## Confidence level used: 0.996666666666667
## [1] "Binding  pairwise CI's"

## Note: Use 'contrast(regrid(object), ...)' to obtain contrasts of back-transformed estimates

##  contrast estimate     SE   df lower.CL upper.CL
##  1 - 2      0.118 0.0522 2458  -0.0355    0.271
##  1 - 3     -0.356 0.0571 2458  -0.5235   -0.188
##  2 - 3     -0.473 0.0557 2458  -0.6372   -0.310
##
## Results are averaged over the levels of: rep.meas
## Note: contrasts are still on the [.: scale
## Confidence level used: 0.996666666666667
# pairwise comparison among time point
fit3 <- manova(respMat[,1:5] ~ tp)
```

```
# summary(fit3)
p <- 5
q3 <- length(unique(tp))
alpha.old <- 0.05
nc3 <- p*q3*(q3-1)/2
alpha.new3 <- alpha.old/nc3

for (i in 1:5){
  w <- c(0, 0, 0, 0, 0)
  w[i] <- 1
  print(paste(vars[i], " pairwise CI's"))
  cont <- contrast(emmeans(fit3, "tp", weights = w), "pairwise")
  bb <- confint(cont, level = 1 - alpha.new3, adj = "none")
  print(bb)
}
```

```
## [1] "L_CDR3  pairwise CI's"

## Note: Use 'contrast(regrid(object), ...)' to obtain contrasts of back-transformed estimates

##  contrast estimate     SE   df lower.CL upper.CL
##  0 - 1     -0.0169 0.0652 2457   -0.222    0.188
##  0 - 2     -0.0287 0.0667 2457   -0.239    0.181
##  0 - 3     -0.0034 0.0764 2457   -0.244    0.237
##  1 - 2     -0.0117 0.0447 2457   -0.153    0.129
##  1 - 3      0.0135 0.0581 2457   -0.169    0.197
##  2 - 3      0.0253 0.0598 2457   -0.163    0.214
##
## Results are averaged over the levels of: rep.meas
## Note: contrasts are still on the [.: scale
## Confidence level used: 0.998333333333333
## [1] "LMuFreq  pairwise CI's"

## Note: Use 'contrast(regrid(object), ...)' to obtain contrasts of back-transformed estimates

##  contrast estimate      SE   df  lower.CL upper.CL
##  0 - 1     0.00853 0.00369 2457 -3.09e-03   0.0202
##  0 - 2     0.01465 0.00378 2457  2.75e-03   0.0265
##  0 - 3     0.01889 0.00432 2457  5.28e-03   0.0325
##  1 - 2     0.00611 0.00253 2457 -1.86e-03   0.0141
##  1 - 3     0.01036 0.00329 2457 -4.12e-06   0.0207
##  2 - 3     0.00424 0.00339 2457 -6.42e-03   0.0149
##
## Results are averaged over the levels of: rep.meas
## Note: contrasts are still on the [.: scale
## Confidence level used: 0.998333333333333
## [1] "H_CDR3  pairwise CI's"

## Note: Use 'contrast(regrid(object), ...)' to obtain contrasts of back-transformed estimates

##  contrast estimate    SE   df lower.CL upper.CL
##  0 - 1      0.489 0.231 2457   -0.238    1.217
##  0 - 2      0.194 0.237 2457   -0.550    0.939
##  0 - 3     -0.608 0.271 2457   -1.460    0.244
##  1 - 2     -0.295 0.159 2457   -0.795    0.204
##  1 - 3     -1.097 0.206 2457   -1.746   -0.448
##  2 - 3     -0.802 0.212 2457   -1.469   -0.134
```

```
## 
## Results are averaged over the levels of: rep.meas
## Note: contrasts are still on the [.: scale
## Confidence level used: 0.998333333333333
## [1] "HMuFreq  pairwise CI's"

## Note: Use 'contrast(regrid(object), ...)' to obtain contrasts of back-transformed estimates

##  contrast estimate      SE   df  lower.CL upper.CL
##  0 - 1     0.00870 0.00288 2457 -0.000379   0.0178
##  0 - 2     0.01872 0.00295 2457  0.009434   0.0280
##  0 - 3     0.02356 0.00338 2457  0.012930   0.0342
##  1 - 2     0.01002 0.00198 2457  0.003796   0.0163
##  1 - 3     0.01486 0.00257 2457  0.006768   0.0230
##  2 - 3     0.00484 0.00265 2457 -0.003491   0.0132
## 
## Results are averaged over the levels of: rep.meas
## Note: contrasts are still on the [.: scale
## Confidence level used: 0.998333333333333
## [1] "Binding  pairwise CI's"

## Note: Use 'contrast(regrid(object), ...)' to obtain contrasts of back-transformed estimates

##  contrast estimate     SE   df lower.CL upper.CL
##  0 - 1     0.00161 0.0689 2457  -0.2151    0.218
##  0 - 2    -1.06381 0.0704 2457  -1.2855   -0.842
##  0 - 3    -0.76734 0.0806 2457  -1.0211   -0.514
##  1 - 2    -1.06542 0.0472 2457  -1.2141   -0.917
##  1 - 3    -0.76895 0.0614 2457  -0.9622   -0.576
##  2 - 3     0.29647 0.0632 2457   0.0976    0.495
## 
## Results are averaged over the levels of: rep.meas
## Note: contrasts are still on the [.: scale
## Confidence level used: 0.998333333333333
```
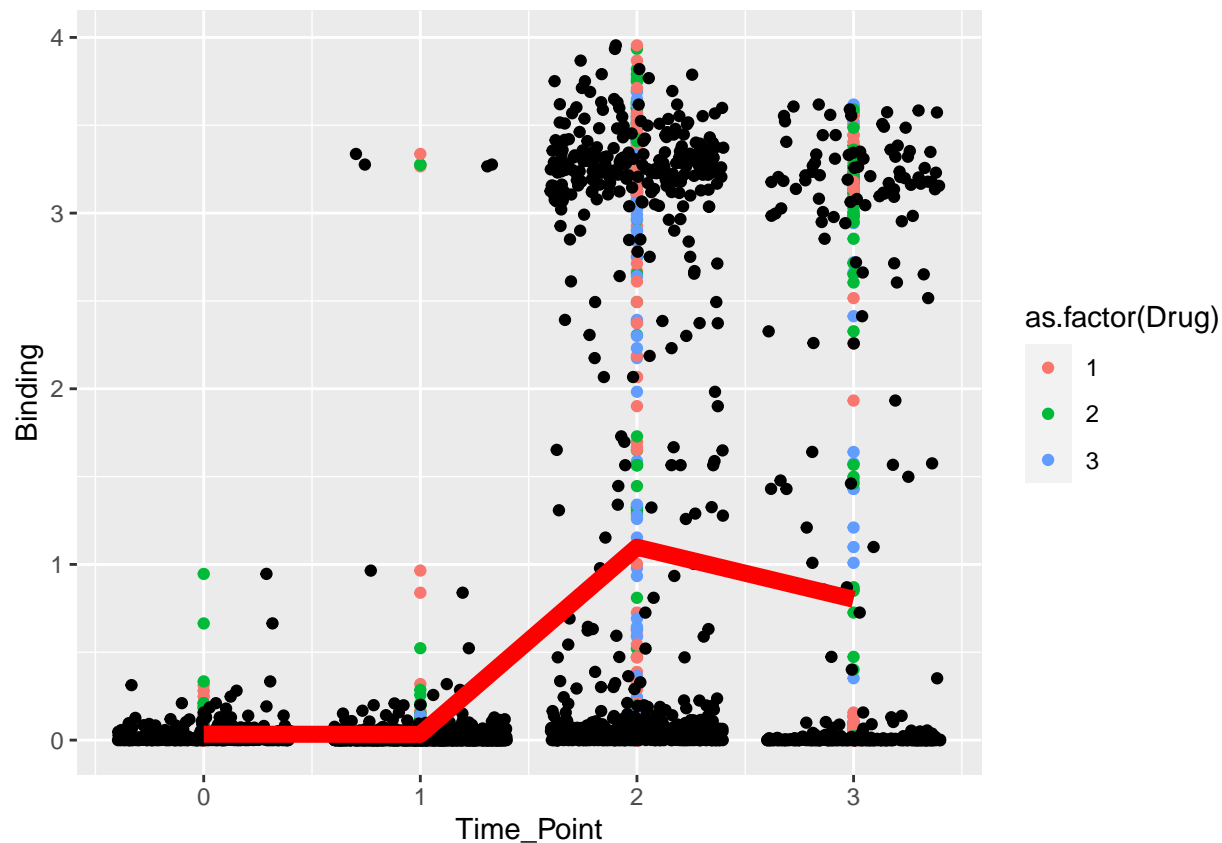
Longitudinal Data Analysis

- check assumption of compound symmetry or equal variance

First we don't consider treatments but only plot the mean trend over time.

$$Y_{ij} = \beta_0 + \beta_1 Time_{ij} + e_{ij}$$

```r
# simply connects the mean of each time point
ggplot(Data3, aes(x = Time_Point, y = Binding)) + geom_point(aes(color = as.factor(Drug))) + geom_jitte
```

Here we use Binding as the only response. Prdictors: `Drug`.
Random effect for both intercept and slope.

$$Y_{ij} = \beta_0 + \beta_1 t_{ij} + b_{0i} + b_{1i} + e_{ij}$$

```
lda <- lme(fixed = Binding ~ Time_Point + Drug,
           random = ~ Time_Point | MonkeyID, data = Data3, method = "REML")
summary(lda)
```

```
## Linear mixed-effects model fit by REML
##   Data: Data3
##        AIC      BIC     logLik
##    6738.894 6779.552 -3362.447
##
## Random effects:
##  Formula: ~Time_Point | MonkeyID
##  Structure: General positive-definite, Log-Cholesky parametrization
##              StdDev      Corr
## (Intercept) 0.6043473 (Intr)
## Time_Point  0.5938004 -0.97
## Residual    0.9330025
##
## Fixed effects: Binding ~ Time_Point + Drug
##                   Value  Std.Error   DF    t-value p-value
## (Intercept) -0.5671081 0.21736533 2443 -2.609009  0.0091
## Time_Point   0.6404275 0.18256794 2443  3.507886  0.0005
```

```
## Drug          0.0414165 0.05945489   18  0.696604  0.4949
##   Correlation:
##            (Intr) Tm_Pnt
## Time_Point -0.847
## Drug       -0.499  0.007
##
## Standardized Within-Group Residuals:
##         Min          Q1         Med          Q3         Max
## -3.52231270 -0.51818636 -0.09479562  0.01991047  3.61431329
##
## Number of Observations: 2464
## Number of Groups: 20
```