MoTrPAC Animal data: analysis of the phenotypic data

```
# Set the working directory to the folder with the data
# setwd("/Users/David/Desktop/MoTrPAC/march_2019/pheno_data/PASS1A.6M-RLS 0,01/3-Data Sets/")
setwd("/Users/David/Desktop/MoTrPAC/april_2019/DMAQC_Transfer_Pass_1A.6M_1/3-Data_Sets/")
all_csvs = list.files(".") # get all files in dir
# read all files
csv_data = list()
for(fname in all_csvs){
  csv_data[[fname]] = read.csv(fname,stringsAsFactors = F)
}# sapply(csv_data,dim) # check the dimensions of the different datasets
```

Acute tests: basic statistics

```
# Get the acute test data
ac_test_data = csv_data[[which(grepl("Acute.Test",names(csv_data)))]]
dim(ac_test_data)
```

```
## [1] 216  27
```

```
# check the time differences between start and end
test_times = as.difftime(ac_test_data$t_complete) - as.difftime(ac_test_data$t_start)
# table of the values: all except for on are 0.5 hours
table(test_times)
```

```
## test_times
## 0.466666666666667               0.5
##                 1               215
```

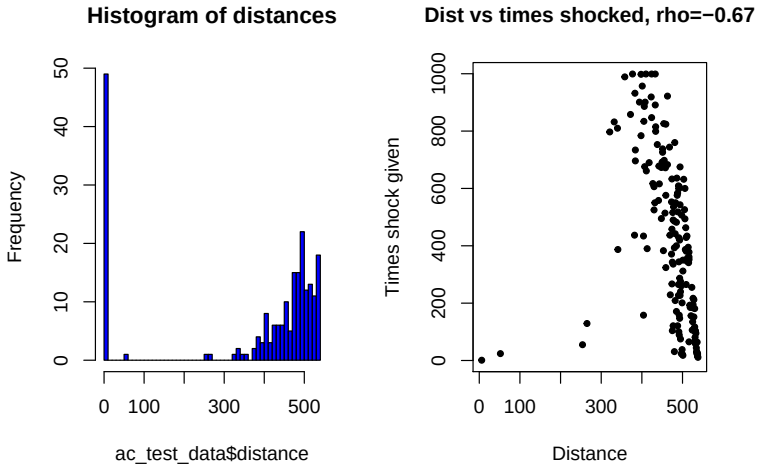
```
# Get the comment of the sample that is not 0.5h
ac_test_data[test_times!=0.5,"comments"]
```

```
## [1] "Treadmill stopped 28:49 (mm:ss) into the acute bout due to problems with the other rat on the sa
```

Nest, we analyze the distances. We hypothesized that these are a function of the shocks given or "errors" in the process.

```
# convert the shock lengths to numbers (seconds)
parse_shocktime<-function(x){
  arr = strsplit(x,split=":")[[1]]
  if(length(arr)<2){return(NA)}
  return(as.numeric(arr[1])*60+as.numeric(arr[2]))
}
tmp_x = ac_test_data$howlongshock
tmp_x = sapply(tmp_x, parse_shocktime)
ac_test_data$howlongshock = tmp_x
rm(tmp_x)

par(mfrow=c(1,2))
# histogram of distances
hist(ac_test_data$distance,col="blue",breaks=50,main = "Histogram of distances")

# Correlation between distance and number of shocks
# Get the indices of the samples with shock information -
```

```r
# these the animals that did the acute test
timesshock_inds = !is.na(ac_test_data$timesshock)
# create a new dataframe with the selected animals
trained_animals_data = ac_test_data[timesshock_inds,]
sp_corr = cor(trained_animals_data$distance,
              trained_animals_data$timesshock,method="spearman")
plot(trained_animals_data$distance,trained_animals_data$timesshock,
     main=paste("Dist vs times shocked, rho=",format(sp_corr,digits = 2),sep=""),
     pch=20,ylab="Times shock given",xlab="Distance",cex.main=1.1)
```




```r
# A "smarter" analysis: regression of the distance using shock info
dist_lm  = lm(distance~timesshock+howlongshock+weight+days_start,
              data=trained_animals_data)
# Summary of the model, points to take: high R^2, significance of
# the features
summary(dist_lm)
```

```
##
## Call:
## lm(formula = distance ~ timesshock + howlongshock + weight +
##     days_start, data = trained_animals_data)
##
## Residuals:
##     Min      1Q  Median      3Q     Max
## -412.69   -1.46    6.48   11.87   70.92
##
## Coefficients:
##               Estimate Std. Error t value Pr(>|t|)
## (Intercept)  548.46424   14.11730  38.851  < 2e-16 ***
## timesshock     0.02645    0.01301   2.033   0.0437 *
## howlongshock  -0.29488    0.01983 -14.871  < 2e-16 ***
## weight        -0.20455    0.04091  -5.000 1.47e-06 ***
## days_start     0.21137    0.15813   1.337   0.1832
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 41.24 on 163 degrees of freedom
## Multiple R-squared:  0.6793, Adjusted R-squared:  0.6714
## F-statistic: 86.32 on 4 and 163 DF,  p-value: < 2.2e-16
```

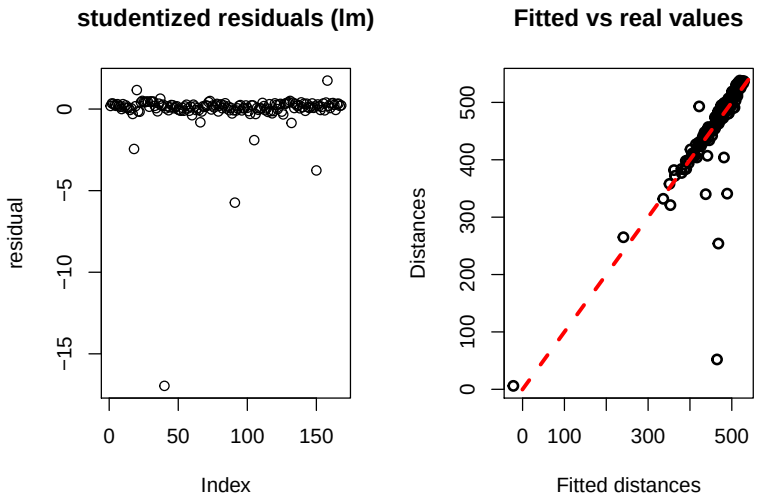
```
# We have some clear outliers:
library(MASS)
par(mfrow=c(1,2))
plot(studres(dist_lm),main="studentized residuals (lm)",ylab="residual")
# Select the top outliers and look at their comments
outliers = abs(studres(dist_lm)) > 2
# how many outliers have we selected?
sum(outliers)
```

```
## [1] 4
```

```
# their comments:
trained_animals_data[outliers,"comments"]
```

```
## [1] ""
## [2] "Halfway through the acute test, the shocker grid malfunctioned and would not shut off even when
## [3] "The shocker grid started to malfunction at time point 18:25 of the run. The distance ran, time
## [4] "Shocker grid malfunctioned. Distance ran, time spent on shocker grid, and number of times rat wa
```

```
# Plot the fitted values of the linear regression vs.
# the true distances
plot(dist_lm$fitted.values,trained_animals_data$distance,lwd=2,
     main="Fitted vs real values",ylab="Distances",xlab="Fitted distances")
abline(0,1,col="red",lty=2,lwd=3)
```



Site comparison

```
# Load additional information about the animals
registr_data = csv_data[[which(grepl("Regist",names(csv_data)))]]
rownames(registr_data) = as.character(registr_data$participantGUID)
# make the rownames in the test data comparable
rownames(trained_animals_data) = trained_animals_data$participantGUID
# add sex to the trained animal data data frame
sex_key = c("Female","Male")
trained_animals_data$sex = sex_key[registr_data[rownames(trained_animals_data),"sex"]]

# Map site Ids to their names
site_names = c("910"="Joslin","930"="Florida")
```

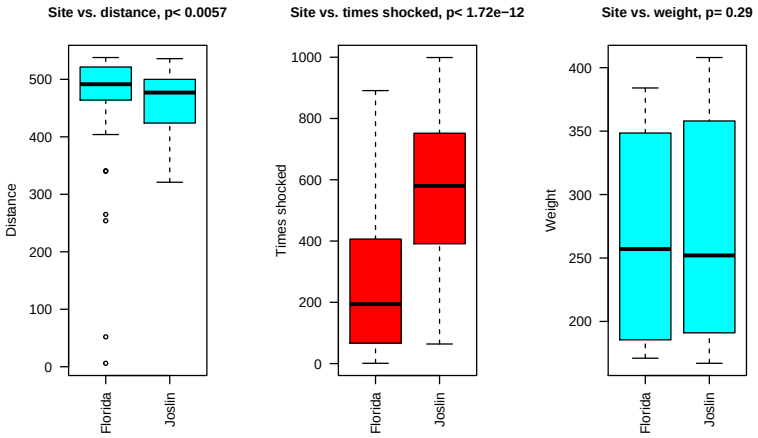
```
trained_animals_data$site = site_names[as.character(trained_animals_data$siteID)]

# Sanity check: the numbers should be the same for both sites
table(ac_test_data$siteID)
```

```
##
## 910 930
## 108 108
```

```
table(trained_animals_data$site,trained_animals_data$sex)
```

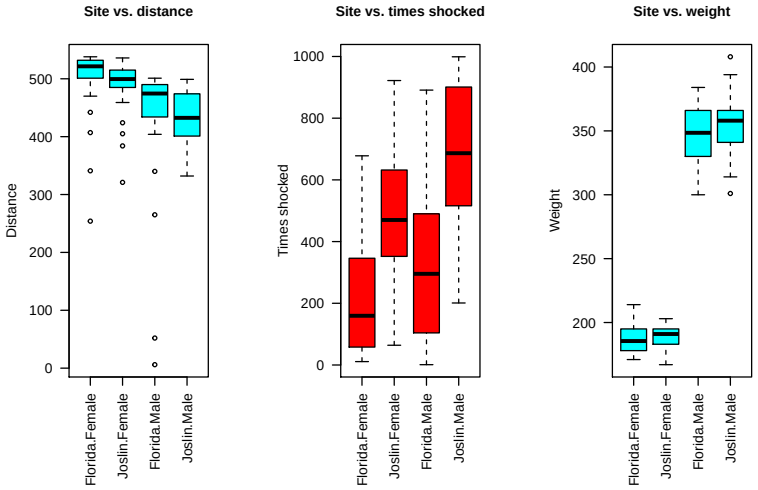
```
##
##           Female Male
##   Florida     42   42
##   Joslin      42   42
```

```
run_wilcox<-function(x1,x2){
  return(wilcox.test(x1[x2==x2[1]],x1[x2!=x2[1]])$p.value)
}
# Compare the distances, shocks, and weight
par(mfrow=c(1,3),mar=c(10,4,4,4))
# Site only
p_dist = run_wilcox(trained_animals_data$distance,trained_animals_data$site)
boxplot(distance~site,data=trained_animals_data,col="cyan",ylab="Distance",
        main=paste("Site vs. distance, p<",format(p_dist,digits = 2)),
        cex.main=1,las=2)
p_timesshock = run_wilcox(trained_animals_data$timesshock,trained_animals_data$site)
boxplot(timesshock~site,data=trained_animals_data,col="red",ylab="Times shocked",
        main=paste("Site vs. times shocked, p<",format(p_timesshock,digits = 3)),
        cex.main=1,las=2)
p_w = run_wilcox(trained_animals_data$weight,trained_animals_data$site)
boxplot(weight~site,data=trained_animals_data,col="cyan",ylab="Weight",
        main=paste("Site vs. weight, p=",format(p_w,digits = 2)),
        cex.main=1,las=2)
```




```
# Site and sex
par(mfrow=c(1,3),mar=c(10,4,4,4))
boxplot(distance~site+sex,data=trained_animals_data,col="cyan",ylab="Distance",
        main="Site vs. distance",cex.main=1,las=2)
boxplot(timesshock~site+sex,data=trained_animals_data,col="red",ylab="Times shocked",
        main="Site vs. times shocked",cex.main=1,las=2)
```

```
boxplot(weight~site+sex,data=trained_animals_data,col="cyan",ylab="Weight",
        main="Site vs. weight",cex.main=1,las=2)
```




```
# Regress time shocked and distance vs. site and sex
summary(lm(timesshock~site+sex,data=trained_animals_data))
```

```
##
## Call:
## lm(formula = timesshock ~ site + sex, data = trained_animals_data)
##
## Residuals:
##     Min      1Q  Median      3Q     Max
## -453.06 -167.17  -11.96  151.83  543.99
##
## Coefficients:
##             Estimate Std. Error t value Pr(>|t|)
## (Intercept)   197.92      31.02   6.380 1.72e-09 ***
## siteJoslin    307.05      35.82   8.572 7.02e-15 ***
## sexMale       149.10      35.82   4.162 5.06e-05 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 232.1 on 165 degrees of freedom
## Multiple R-squared:  0.355,  Adjusted R-squared:  0.3471
## F-statistic:  45.4 on 2 and 165 DF,  p-value: < 2.2e-16
```

```
summary(lm(distance~site+sex,data=trained_animals_data))
```

```
##
## Call:
## lm(formula = distance ~ site + sex, data = trained_animals_data)
##
## Residuals:
##     Min      1Q  Median      3Q     Max
## -435.20  -10.88   15.44   33.70   67.24
##
## Coefficients:
##             Estimate Std. Error t value Pr(>|t|)
## (Intercept)  501.780      8.745  57.381  < 2e-16 ***
```

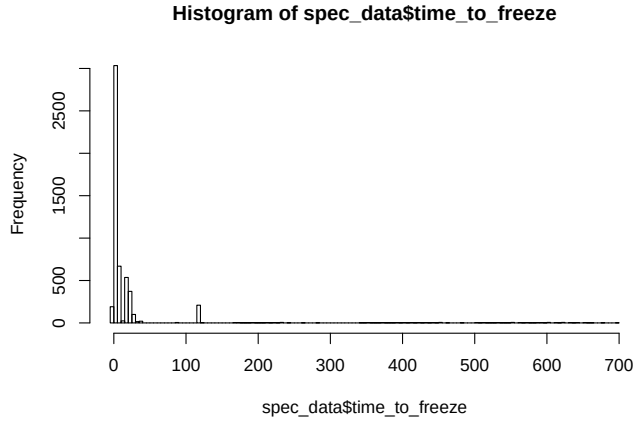
```
## siteJoslin    -9.440     10.097  -0.935    0.351
## sexMale      -60.583     10.097  -6.000 1.21e-08 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 65.44 on 165 degrees of freedom
## Multiple R-squared:  0.1827, Adjusted R-squared:  0.1727
## F-statistic: 18.44 on 2 and 165 DF,  p-value: 5.937e-08
```

Biospecimen data

```
# Analysis of biospecimen data
spec_data = csv_data[[which(grepl("Specimen.Processing.csv",names(csv_data)))]]
# Parse the times and compute the difference between the freeze time and
# the collection time
time_to_freeze1 = as.difftime(spec_data$t_freeze,units = "mins") -
  as.difftime(spec_data$t_collection,units="mins")
# For some samples we have the edta spin time instead of the collection
# time, use these when there are no other options
time_to_freeze2 = as.difftime(spec_data$t_freeze,units = "mins") -
  as.difftime(spec_data$t_edtaspin,units="mins")
time_to_freeze = time_to_freeze1
# Fill in the NAs by taking the time between the edta spin and the freeze
table(is.na(time_to_freeze1),is.na(time_to_freeze2))
```

```
##
##         FALSE TRUE
##   FALSE     0 4345
##   TRUE   1047    5
```

```
time_to_freeze[is.na(time_to_freeze1)] = time_to_freeze2[is.na(time_to_freeze1)]
spec_data$time_to_freeze = as.numeric(time_to_freeze)
hist(spec_data$time_to_freeze,breaks=100)
```

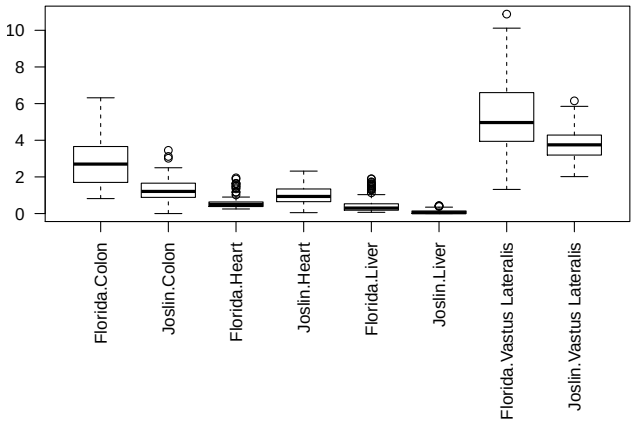



```
# Add site by name
site_names = c("910"="Joslin","930"="Florida")
spec_data$site = site_names[as.character(spec_data$siteid)]

inds = !is.na(time_to_freeze1)
inds = grepl("adipose",spec_data$sampletypedescription,ignore.case = T)
```

```r
inds = grepl("heart",spec_data$sampletypedescription,ignore.case = T) |
  grepl("liver",spec_data$sampletypedescription,ignore.case = T) |
  grepl("colon",spec_data$sampletypedescription,ignore.case = T) |
  grepl("vastus",spec_data$sampletypedescription,ignore.case = T)
# Here we use an interaction term and not addition as the R^2 is >2 times
# greater this way
summary(lm(time_to_freeze~sampletypedescription:site,data=spec_data[inds,]))
```

```
##
## Call:
## lm(formula = time_to_freeze ~ sampletypedescription:site, data = spec_data[inds,
##     ])
##
## Residuals:
##     Min      1Q  Median      3Q     Max
## -4.0282 -0.3597 -0.0855  0.3086  5.5385
##
## Coefficients: (1 not defined because of singularities)
##                                                    Estimate Std. Error
## (Intercept)                                          3.8043     0.0871
## sampletypedescriptionColon:siteFlorida              -1.0796     0.1235
## sampletypedescriptionHeart:siteFlorida              -3.1998     0.1235
## sampletypedescriptionLiver:siteFlorida              -3.3148     0.1235
## sampletypedescriptionVastus Lateralis:siteFlorida    1.5405     0.1235
## sampletypedescriptionColon:siteJoslin               -2.4838     0.1232
## sampletypedescriptionHeart:siteJoslin               -2.7843     0.1232
## sampletypedescriptionLiver:siteJoslin               -3.7188     0.1232
## sampletypedescriptionVastus Lateralis:siteJoslin         NA         NA
##                                                    t value Pr(>|t|)
## (Intercept)                                         43.679   <2e-16 ***
## sampletypedescriptionColon:siteFlorida              -8.744   <2e-16 ***
## sampletypedescriptionHeart:siteFlorida             -25.917   <2e-16 ***
## sampletypedescriptionLiver:siteFlorida             -26.848   <2e-16 ***
## sampletypedescriptionVastus Lateralis:siteFlorida   12.478   <2e-16 ***
## sampletypedescriptionColon:siteJoslin              -20.165   <2e-16 ***
## sampletypedescriptionHeart:siteJoslin              -22.604   <2e-16 ***
## sampletypedescriptionLiver:siteJoslin              -30.191   <2e-16 ***
## sampletypedescriptionVastus Lateralis:siteJoslin       NA       NA
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.9051 on 852 degrees of freedom
## Multiple R-squared:  0.7885, Adjusted R-squared:  0.7867
## F-statistic: 453.6 on 7 and 852 DF,  p-value: < 2.2e-16
```

```r
par(mar=c(10,2,2,2))
boxplot(time_to_freeze~site:sampletypedescription,data=spec_data[inds,],
        ylab="Time to freeze",las=2)
```


10
8
6
4
2
0
Florida.Colon
Joslin.Colon
Florida.Heart
Joslin.Heart
Florida.Liver
Joslin.Liver
Florida.Vastus Lateralis
Joslin.Vastus Lateralis