Plasma CORT

Kyle Emerson

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## R Markdown for Plasma CORT

library(lme4)

## Warning: package 'lme4' was built under R version 4.1.3

## Loading required package: Matrix

## Warning: package 'Matrix' was built under R version 4.1.3

library(car)

## Warning: package 'car' was built under R version 4.1.3

## Loading required package: carData

## Warning: package 'carData' was built under R version 4.1.3

library(readr)

## Warning: package 'readr' was built under R version 4.1.3

library(moments)

## Warning: package 'moments' was built under R version 4.1.3

library(psych)

## Warning: package 'psych' was built under R version 4.1.3

##   
## Attaching package: 'psych'

## The following object is masked from 'package:car':  
##   
## logit

library(pastecs)  
library(ggplot2)

## Warning: package 'ggplot2' was built under R version 4.1.3

##   
## Attaching package: 'ggplot2'

## The following objects are masked from 'package:psych':  
##   
## %+%, alpha

library(dplyr)

## Warning: package 'dplyr' was built under R version 4.1.3

##   
## Attaching package: 'dplyr'

## The following objects are masked from 'package:pastecs':  
##   
## first, last

## The following object is masked from 'package:car':  
##   
## recode

## The following objects are masked from 'package:stats':  
##   
## filter, lag

## The following objects are masked from 'package:base':  
##   
## intersect, setdiff, setequal, union

library(emmeans)  
  
file.choose()

## [1] "C:\\R\\Aim 3 - Stress and the Microbiota\\Plasma-CORT.docx"

df <- read.csv("C:\\Users\\kjeme\\OneDrive\\Desktop\\Woodley Lab\\Aim 3 - CORT & the Microbiota\\Aim 3 (Restarted)\\KE Data FIles\\Aim 3 - Plasma CORT Assay\\Aim 3 Plasma Cort.csv")  
df <- df[-c(29),]  
#Removed tadpole that had fully formed forelimbs  
  
df$Water\_Trtmt = factor(df$Water\_Trtmt)  
df$Stressor = factor(df$Stressor)  
df$Replicate = factor(df$Replicate)  
df$Trtmt\_Combo = factor(df$Trtmt\_Combo)  
df$Exp\_Days = factor(df$Exp\_Days)  
df$Gosner\_Stage = as.numeric(df$Gosner\_Stage)  
  
shapiro.test(df$Corticosterone)

##   
## Shapiro-Wilk normality test  
##   
## data: df$Corticosterone  
## W = 0.64055, p-value = 1.763e-09

Fail.

leveneTest(df$Corticosterone, df$Trtmt\_Combo, center = mean, na.rm = TRUE)

## Levene's Test for Homogeneity of Variance (center = mean: TRUE)  
## Df F value Pr(>F)   
## group 5 2.5194 0.04448 \*  
## 41   
## ---  
## Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

Fail.

shapiro.test(df$Log\_CORT)

##   
## Shapiro-Wilk normality test  
##   
## data: df$Log\_CORT  
## W = 0.97704, p-value = 0.476

Pass with log.

leveneTest(df$Log\_CORT, df$Trtmt\_Combo, center = mean, na.rm = TRUE)

## Levene's Test for Homogeneity of Variance (center = mean: TRUE)  
## Df F value Pr(>F)  
## group 5 0.2811 0.9208  
## 41

Pass with log.

CORT\_glmm <- glmer(Log\_CORT~Water\_Trtmt\*Stressor + (1|Replicate),  
 data = df, family = "gaussian")

## Warning in glmer(Log\_CORT ~ Water\_Trtmt \* Stressor + (1 | Replicate), data =  
## df, : calling glmer() with family=gaussian (identity link) as a shortcut to  
## lmer() is deprecated; please call lmer() directly

Anova(CORT\_glmm)

## Analysis of Deviance Table (Type II Wald chisquare tests)  
##   
## Response: Log\_CORT  
## Chisq Df Pr(>Chisq)  
## Water\_Trtmt 0.0048 1 0.9448  
## Stressor 0.7196 2 0.6978  
## Water\_Trtmt:Stressor 0.2186 2 0.8965

Nonsignificant effects on plasma CORT.

emmeans(CORT\_glmm, list(pairwise ~ Stressor), adjust = "tukey")

## NOTE: Results may be misleading due to involvement in interactions

## $`emmeans of Stressor`  
## Stressor emmean SE df lower.CL upper.CL  
## A 0.0139 0.1001 18.7 -0.196 0.2236  
## B -0.1060 0.0979 17.6 -0.312 0.0999  
## C -0.0476 0.0979 17.6 -0.254 0.1584  
##   
## Results are averaged over the levels of: Water\_Trtmt   
## Degrees-of-freedom method: kenward-roger   
## Confidence level used: 0.95   
##   
## $`pairwise differences of Stressor`  
## 1 estimate SE df t.ratio p.value  
## A - B 0.1200 0.140 18.1 0.857 0.6731  
## A - C 0.0615 0.140 18.1 0.440 0.8995  
## B - C -0.0584 0.138 17.6 -0.422 0.9069  
##   
## Results are averaged over the levels of: Water\_Trtmt   
## Degrees-of-freedom method: kenward-roger   
## P value adjustment: tukey method for comparing a family of 3 estimates

No effect of stressor in pairwise comaprisons.

Stress <- c("Vehicle Control", "Predator Cues", "CORT")  
#For labeling X axis  
  
ggplot(df, aes(x= factor(Stressor, level=c('B', 'A','C')), y = Corticosterone, fill = Water\_Trtmt)) +  
 geom\_boxplot(width = 0.5, alpha =0.35) +  
 stat\_boxplot(geom = "errorbar", width = .5) +  
 theme\_classic() +  
 scale\_fill\_manual(values = c("seagreen3", "lightcyan"),  
 name = "Microbial Environment",  
 labels = c("Natural", "Autoclaved")) +  
 labs(x = "Stressor", y = "Plasma Corticosterone (ng/mL)") +  
 theme(aspect.ratio = 1) +  
 scale\_x\_discrete(labels = Stress)+  
 theme(axis.text = element\_text(face = "bold", size = 16)) +  
 theme(axis.title = element\_text(face = "bold", size = 20)) +  
 theme(legend.position = c(.83, .92)) +  
 theme(legend.title = element\_text(size = 14)) +  
 theme(legend.text = element\_text(size = 14))

