temp_manipulation_markdown

Julie Jung

May 2, 2018

Contents

Introduction

Welcome, friends! Please go forth and scroll down to begin your journey though my data analysis for our temperature manipulation experiment. Here I have provided some basic background and performed all analyses used for a manuscript in preparation.

Red-eyed treefrogs, *Agalychnis callidryas* (pictured above as an adult and a juvenile), commonly lay their eggs (pictured below left) on leafy substrate overhanging tropical ponds .

These arboreal embryos can hatch prematurely to escape from egg predators, such as the parrot snake (pictured above right), cued by vibrations in attacks.

A developmental match in the onset of otic function, measured by the vestibulo-ocular reflex (VOR), and mechanosensory-cued hatching (MCH) suggests that inner ear mechanoreceptors mediate this response.

Methods

We manipulated development rate by rearing embryos under three thermal conditions (warm, ambient, cool) to assess consistency in the matched onset of VOR and hatching responses to simulated attack cues.

Then, we employed confocal microscopy of whole otic capsules of embryos preserved just before and after the onset of MCH (about 3 h apart), visualizing F-actin and synapsin.

Hypothesis

We predict that VOR and MCH will appear concurrently in each thermal treatment.

We also believe that several morphometrics of inner ears, including abundance and density of stereociliary bundles and their synaptic connections, will increase across hatching onset.

Statistics

Code preparation

File logistics

First, we will clear the environment and set our working directory: Prep the environment and load in our data

```
rm(list=ls()) #clear environment
setwd('/Users/juliejung/Documents/GitHub/temp_manipulation') #set working directory
```

User Defined Functions

Here we'll define some functions that will help us later. The following function will give us the mode:

```
# gives mode
Mode <- function(x) {
  ux <- unique(x)
  ux[which.max(tabulate(match(x, ux)))]
}
```

Load packages

Here, we install and load packages that we'll need later on in the code. NOTE: uncomment as needed.

```r
# install.packages("stargazer")
# install.packages("knitr")
# install.packages("dplyr")
# install.packages("curl")
# install.packages("sciplot")
# install.packages("ggplot2")
# install.packages("MASS")
# install.packages("multcomp")
# install.packages("AICcmodavg")
# install.packages("car")

library("xlsx")
library("stargazer")
```

```
##
## Please cite as:

##  Hlavac, Marek (2018). stargazer: Well-Formatted Regression and Summary Statistics Tables.

##  R package version 5.2.2. https://CRAN.R-project.org/package=stargazer
```

```r
library("knitr")
library("dplyr")
```

```
##
## Attaching package: 'dplyr'

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

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

```r
library("curl")
library("sciplot")
library("ggplot2")
library("MASS")
```

```
##
## Attaching package: 'MASS'

## The following object is masked from 'package:dplyr':
##
##     select
```

```r
library("multcomp")
```

```
## Loading required package: mvtnorm

## Loading required package: survival

## Loading required package: TH.data

##
## Attaching package: 'TH.data'
```

```
## The following object is masked from 'package:MASS':
##
##     geyser
library("car")
```

```
## Loading required package: carData
```

```
##
## Attaching package: 'car'
```

```
## The following object is masked from 'package:dplyr':
##
##     recode
```

Results

Part I : VOR and MCH appear concurrently in each thermal treatment

```
data<-read.csv(file="HotCold_20180724.csv")
```

Now let's look at the structure of our data, make sure the variables are defined as we want them to be defined, and find the average of Kaylee and Avital's counts.

```
#str(data)

data$Objective<-as.factor(data$Objective)
data$HatchingState<-as.factor(data$HatchingState)
data$Clutch<-as.factor(data$Clutch)

#data$RampDiff<-as.numeric(as.character(data$RampDiff))
#data$R.R2<-as.numeric(as.character(data$R.R2))
#data$LampDiff<-as.numeric(as.character(data$LampDiff))
#data$L.R2<-as.numeric(as.character(data$L.R2))
#data$AverageAmp<-as.numeric(as.character(data$AverageAmp))
#data$AverageR2<-as.numeric(as.character(data$AverageR2))
#data$Stage<-as.numeric(as.character(data$Stage))
#data$Z_first<-as.numeric(as.character(data$Z_first))
#data$Z_last<-as.numeric(as.character(data$Z_last))
#data$N_images_in_sequence<-as.numeric(as.character(data$N_images_in_sequence))
#data$Kaylee_N_hair_cells<-as.numeric(as.character(data$Kaylee_N_hair_cells))
#data$Kaylee_Total_N_of_hair_cells<-as.numeric(as.character(data$Kaylee_Total_N_of_hair_cells))
#data$Kaylee_HC_to_HC_length_um<-as.numeric(as.character(data$Kaylee_HC_to_HC_length_um))
#data$Kaylee_HC_to_HC_surface_area<-as.numeric(as.character(data$Kaylee_HC_to_HC_surface_area))
#data$Kaylee_HC_surface_length_um<-as.numeric(as.character(data$Kaylee_HC_surface_length_um))
#data$Kaylee_HC_surface_area<-as.numeric(as.character(data$Kaylee_HC_surface_area))
#data$Avital_N_hair_cells<-as.numeric(as.character(data$Avital_N_hair_cells))
#data$Avital_Total_N_of_hair_cells<-as.numeric(as.character(data$Avital_Total_N_of_hair_cells))
#data$Avital_HC_to_HC_length_um<-as.numeric(as.character(data$Avital_HC_to_HC_length_um))
#data$Avital_HC_to_HC_surface_area<-as.numeric(as.character(data$Avital_HC_to_HC_surface_area))
#data$Avital_HC_surface_length_um<-as.numeric(as.character(data$Avital_HC_surface_length_um))
#data$Avital_HC_surface_area<-as.numeric(as.character(data$Avital_HC_surface_area))

data$average_N_hair_cells <- rowMeans(data[c('Kaylee_N_hair_cells', 'Avital_N_hair_cells')], na.rm=TRUE)
```

```
data$avg_total_N_hair_cells <- rowMeans(data[c('Avital_Total_N_of_hair_cells', 'Kaylee_Total_N_of_hair_
data$Average_HC_to_HC_surface_area <- rowMeans(data[c('Avital_HC_to_HC_surface_area', 'Kaylee_HC_to_HC_s
data$Average_HC_surface_area <- rowMeans(data[c('Avital_HC_surface_area', 'Kaylee_HC_surface_area')], na

#data$Kaylee_N_synapses<-as.numeric(as.character(data$Kaylee_N_synapses))
#data$Kaylee_Total_N_synapses<-as.numeric(as.character(data$Kaylee_Total_N_synapses))
#data$Avital_N_synapses<-as.numeric(as.character(data$Avital_N_synapses))
#data$Avital_Total_N_synapses<-as.numeric(as.character(data$Avital_Total_N_synapses))
#data$Julie_N_synapses<-as.numeric(as.character(data$Julie_N_synapses))
#data$Julie_Total_N_synapses<-as.numeric(as.character(data$Julie_Total_N_synapses))

data$average_N_synapses <- rowMeans(data[c('Avital_N_synapses', 'Julie_N_synapses', 'Kaylee_N_synapses'

#data$average_total_N_synapses <- rowMeans(data[c('Avital_Total_N_synapses', 'Julie_Total_N_synapses',
#data$Kaylee_SiCE_N_synapses<-as.numeric(as.character(data$Kaylee_SiCE_N_synapses))
```

Here let's subset the data by treatment:

```
Hot<- subset(data, Temperature=="Hot", na.rm=TRUE)
Cold<- subset(data, Temperature=="Cold", na.rm=TRUE)

quantile(Hot$Stage)
```

```
##   0%  25%  50%  75% 100%
##   27   27   28   29   29
```

```
quantile(Cold$Stage)
```

```
##   0%  25%  50%  75% 100%
##   27   28   28   28   29
```

Find avg stages per temp per age group -

```
stage_summary <-
  data %>%
  group_by(Temperature, AgeGroup) %>%
  summarize(count = n(),
            mean = mean(Stage, na.rm=TRUE),
            SD = sd(Stage, na.rm=TRUE),
            SE = sd(Stage, na.rm=TRUE)/sqrt(n())
            )
kable(stage_summary,title="Mean & SD & SE", digits=3)
```

Temperature	AgeGroup	count	mean	SD	SE
Cold	Older	636	28.159	0.366	0.015
Cold	Younger	391	27.568	0.496	0.025
Hot	Older	506	28.457	0.603	0.027
Hot	Younger	254	27.004	0.063	0.004

VOR in all the extra tads measured also :

```
allVORdata<-read.xlsx(file="VOR-Temp manipulation data.xlsx", sheetName="colors")
str(allVORdata)
```

```
## 'data.frame':    162 obs. of  25 variables:
##  $ date         : num  20170719 20170719 20170719 20170719 20170719 ...
##  $ age          : num  3 3 3 3 3 3 3 4 4 4 ...
##  $ time.of.day  : Factor w/ 13 levels "1:00am","1:00pm",..: 2 8 10 10 2 8 10 1 7 9 ...
##  $ hrs.since.ovi: num  85 88 90 90 85 88 90 97 100 102 ...
##  $ clutch       : num  291 291 291 291 292 292 292 312 312 312 ...
##  $ stage        : Factor w/ 6 levels "25","26","27",..: 3 4 4 4 3 1 4 1 2 2 ...
##  $ tube.number  : num  1 3 5 7 2 4 6 8 9 13 ...
##  $ individual   : num  1 1 1 2 1 1 1 1 1 1 ...
##  $ treatment    : Factor w/ 2 levels "cool","warm": 2 2 2 2 2 2 2 1 1 1 ...
##  $ CCW.image.start: Factor w/ 162 levels "1010","1038",..: 34 36 39 41 35 38 40 42 43 48 ...
##  $ CCW.image.end  : Factor w/ 162 levels "1023","1053",..: 33 35 37 41 34 36 39 42 43 48 ...
##  $ CW.image.start : Factor w/ 162 levels "1024","1054",..: 33 35 37 41 34 36 38 42 43 48 ...
##  $ CW.image.end   : Factor w/ 162 levels "1008","1036",..: 34 37 39 41 35 38 40 42 44 48 ...
##  $ hatched      : Factor w/ 3 levels "no","possible",..: 1 1 NA 3 1 1 3 1 1 NA ...
##  $ visual.VOR   : Factor w/ 7 levels "0","1","2","no",..: 4 4 NA NA 4 1 2 4 4 NA ...
##  $ sample       : Factor w/ 63 levels "A- Green -c371-88",..: NA NA NA 53 NA 49 54 NA NA NA ...
##  $ RampDiff     : num  NA NA NA 15.8 NA ...
##  $ R.R2         : num  NA NA NA 0.702 NA ...
##  $ LampDiff     : num  NA NA NA 14.4 NA ...
##  $ L.R2         : num  NA NA NA 0.668 NA ...
##  $ Average.Amp  : num  NA NA NA 15.1 NA ...
##  $ Visual.Check : Factor w/ 2 levels "ok","x": NA NA NA 1 NA 2 1 NA NA NA ...
##  $ Corrected.Avg: num  NA NA NA 15.1 NA ...
##  $ Average.R2   : Factor w/ 72 levels "-0.0154410872452778",..: NA NA NA 50 NA 2 61 NA NA NA ...
##  $ notes        : Factor w/ 21 levels "delayed to start hatching???",..: NA NA NA NA NA NA NA NA N
```

```r
nonNAs<-subset(allVORdata, (!is.na(allVORdata[,21])))

# Not_corrected_VOR_summary <-
#   nonNAs %>%
#   group_by(treatment, hatched) %>%
#   summarize(count = n(),
#             mean = mean(Average.Amp, na.rm=T),
#             SD = sd(Average.Amp, na.rm=T),
#             SE = sd(Average.Amp, na.rm=T)/sqrt(n())
#             )
# kable(VOR_summary,title="Mean & SD & SE", digits=3)


VOR_summary <-
  nonNAs %>%
  group_by(treatment, hatched) %>%
  summarize(count = n(),
            mean = mean(Corrected.Avg, na.rm=T),
            SD = sd(Corrected.Avg, na.rm=T),
            SE = sd(Corrected.Avg, na.rm=T)/sqrt(n())
            )
kable(VOR_summary,title="Mean & SD & SE", digits=3)
```

treatment	hatched	count	mean	SD	SE
cool	no	26	5.527	7.868	1.543
cool	yes	25	14.926	11.083	2.217
warm	no	12	5.400	6.827	1.971

treatment	hatched	count	mean	SD	SE
warm	yes	12	15.660	7.368	2.127

```
# count = N of individuals in each treatment
```

```
VOR_summary_cold<-VOR_summary[c(1,2),]
VOR_summary_hot<-VOR_summary[c(3,4),]
```

```
ggplot(data=VOR_summary, aes(x=hatched, y=mean), color="Temperature") +
  geom_point(data=VOR_summary_cold, size=3, color="dodgerblue2") +
  geom_errorbar(data=VOR_summary_cold, aes(ymin=mean-SE, ymax=mean+SE), color="dodgerblue2", width=0.05)
  geom_point(data=VOR_summary_hot, size=3, color="red") +
  geom_errorbar(data=VOR_summary_hot, aes(ymin=mean-SE, ymax=mean+SE), color="red", width=0.05)+
  theme_bw(20)+
      theme(panel.grid.major = element_blank(),
          panel.grid.minor = element_blank())+
  ylab("\n VOR amplitude (°)")+
  xlab("Hatched?")
```
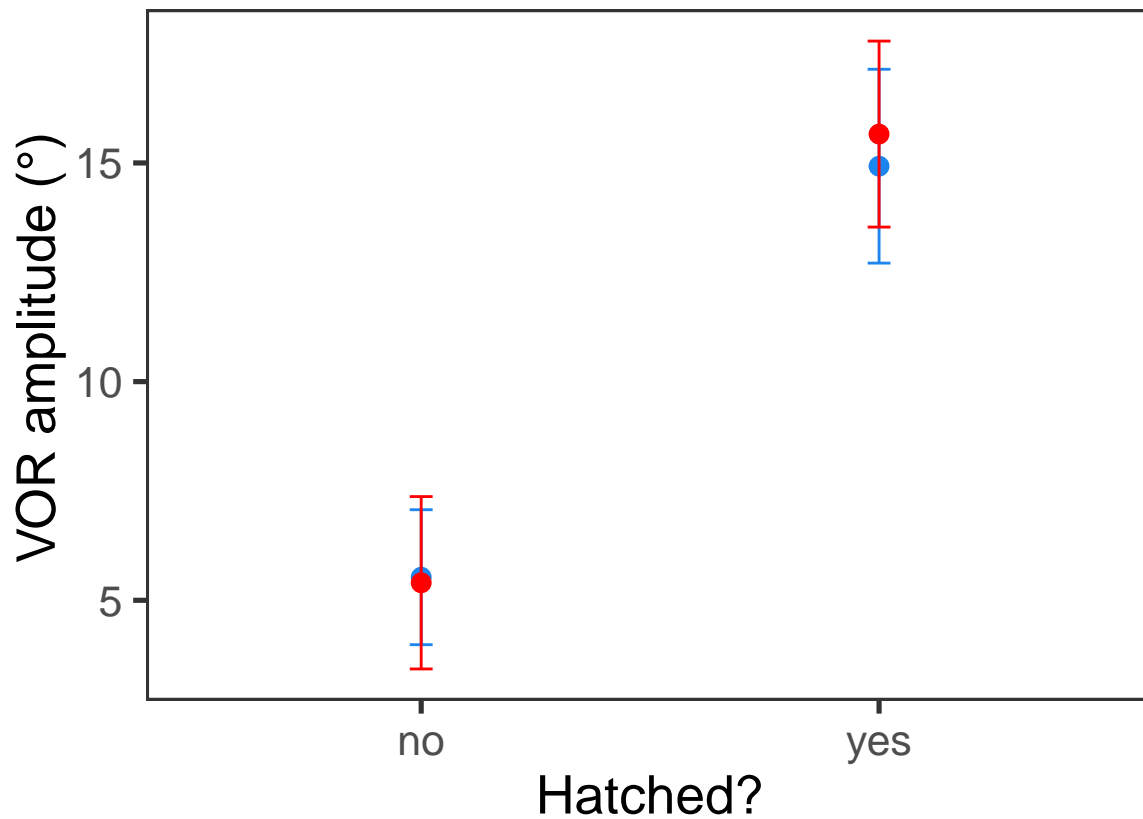

Part II : Compare HC depths/widths between younger and older

Read in HC data & summarize in a table

Next, we want to read in the relevant datafile.

```r
HC_cold_younger<-read.xlsx(file="widthHCcombined.xlsx", sheetName="ColdYounger")
HC_cold_older<-read.xlsx(file="widthHCcombined.xlsx", sheetName="ColdOlder")
HC_hot_younger<-read.xlsx(file="widthHCcombined.xlsx", sheetName="HotYounger")
HC_hot_older<-read.xlsx(file="widthHCcombined.xlsx", sheetName="HotOlder")
```

Add a column for Treatment at the end of each dataframe

```r
HC_cold_younger$Temperature<-"Cold"
HC_cold_older$Temperature<-"Cold"
HC_hot_younger$Temperature<-"Hot"
HC_hot_older$Temperature<-"Hot"

HC_cold_younger$AgeGroup<-"Younger"
HC_cold_older$AgeGroup<-"Older"
HC_hot_younger$AgeGroup<-"Younger"
HC_hot_older$AgeGroup<-"Older"
```

Let's combine the dataframes into 1 comprehensive one!

```r
HC_depth_combined<- rbind(HC_cold_younger, HC_cold_older, HC_hot_younger, HC_hot_older)
```

```r
HC_depth_summary <-
  HC_depth_combined %>%
  group_by(Temperature, AgeGroup) %>%
  summarize(count = n(),
            mean = mean(HC_width_in_um, na.rm=TRUE),
            SD = sd(HC_width_in_um, na.rm=TRUE),
            SE = sd(HC_width_in_um, na.rm=TRUE)/sqrt(n())
            )
kable(HC_depth_summary,title="Mean & SD & SE", digits=3)
```

Temperature	AgeGroup	count	mean	SD	SE
Cold	Older	474	6.747	2.854	0.131
Cold	Younger	344	5.674	2.313	0.125
Hot	Older	409	7.291	2.736	0.135
Hot	Younger	296	5.216	3.253	0.189

```r
mean(HC_depth_summary$mean) #why we measured HCs every 8um
```

```
## [1] 6.232106
```

Plotting hair widths/depths

Make a plot for the hot and cold temps:

```r
HC_depth_summary$AgeGroup <- ordered(HC_depth_summary$AgeGroup, levels=c("Younger", "Older"))
Cold_HC_depth_summary<-HC_depth_summary[1:2,]
Cold_HC_depth_summary$AgeGroup <- ordered(Cold_HC_depth_summary$AgeGroup, levels=c("Younger", "Older"))
Hot_HC_depth_summary<-HC_depth_summary[3:4,]
Hot_HC_depth_summary$AgeGroup <- ordered(Hot_HC_depth_summary$AgeGroup, levels=c("Younger", "Older"))

ggplot(data=HC_depth_summary, aes(x=AgeGroup, y=mean), color="Temperature") +
  geom_point(data=Cold_HC_depth_summary, size=3, color="blue") +
  geom_errorbar(data=Cold_HC_depth_summary, aes(ymin=mean-SE, ymax=mean+SE), color="blue", width=0.05)+
```

```
geom_point(data=Hot_HC_depth_summary, size=3, color="red") +
geom_errorbar(data=Hot_HC_depth_summary, aes(ymin=mean-SE, ymax=mean+SE), color="red", width=0.05)+
theme_bw(20)+
    theme(panel.grid.major = element_blank(),
      panel.grid.minor = element_blank())+
ylab("Hair Cell Width (µm)\n")+
xlab("\n Age Group")
```
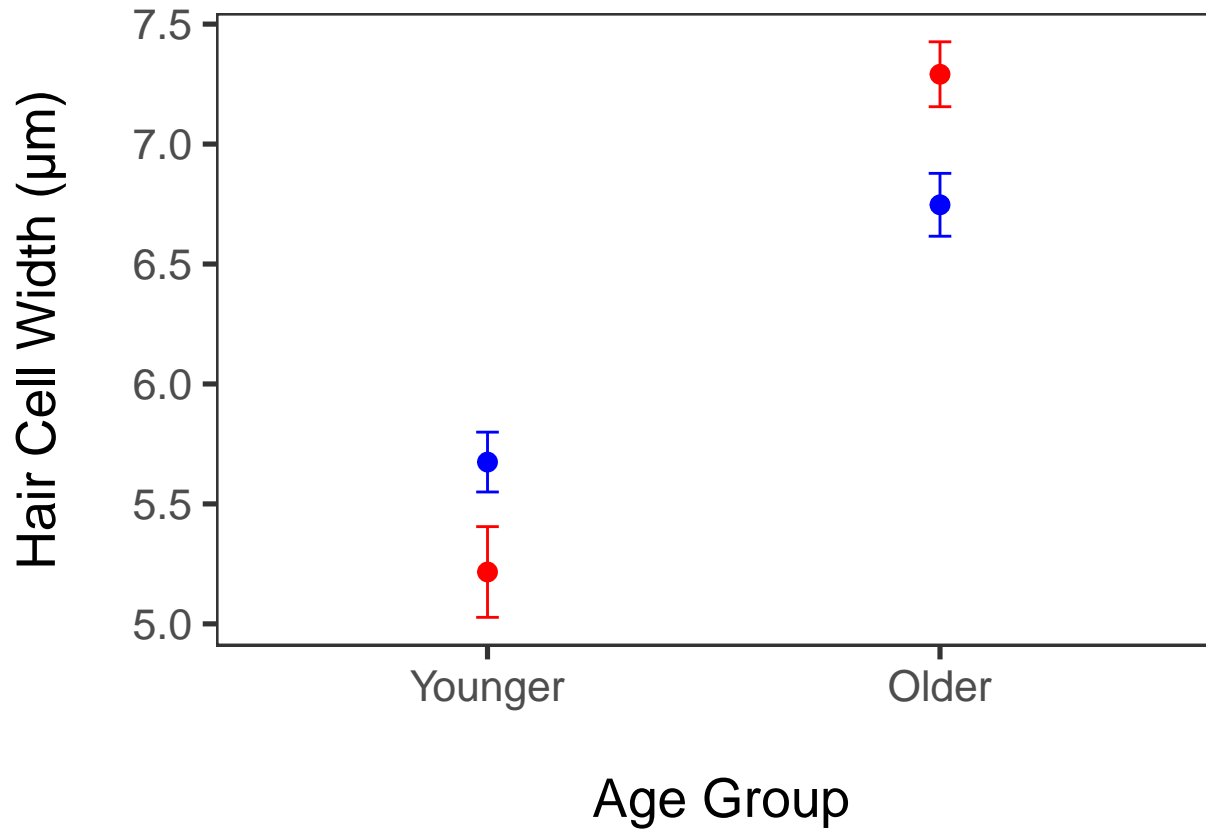

Stats for significant differences between younger and older (in hot and cold dataset)

```
hist(HC_depth_combined$HC_width_in_um) #nonnormal
```

Histogram of HC_depth_combined$HC_width_in_um
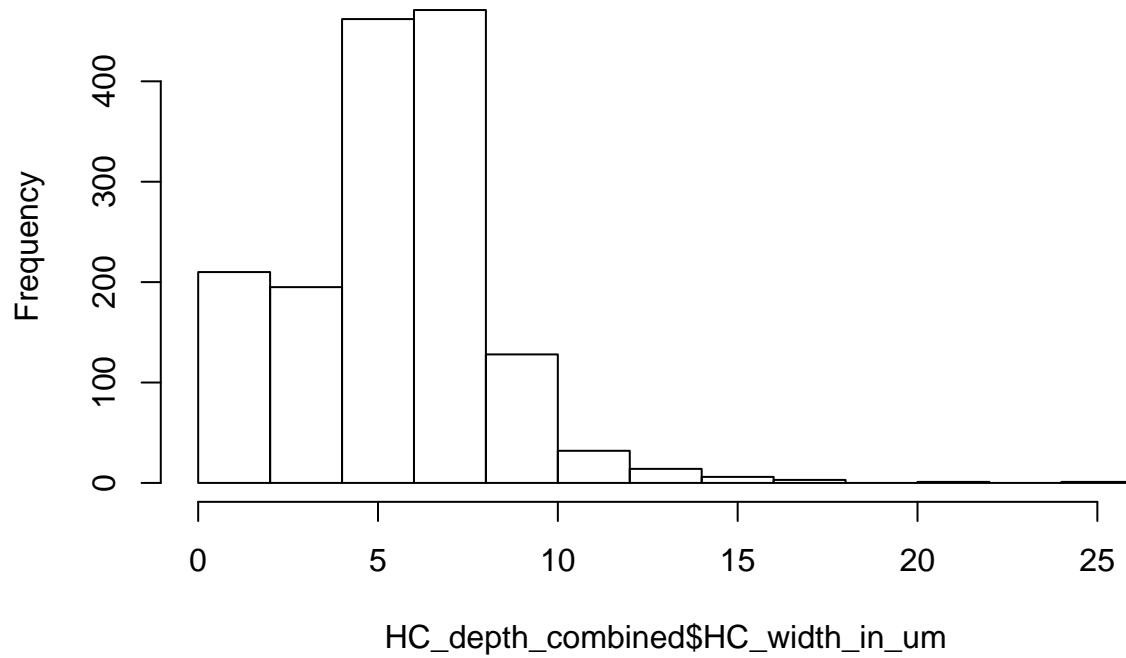

```r
shapiro.test(HC_depth_combined$HC_width_in_um) #nonnormal
```

```
## 
##  Shapiro-Wilk normality test
## 
## data:  HC_depth_combined$HC_width_in_um
## W = 0.92379, p-value < 2.2e-16
```

```r
hist(log(HC_depth_combined$HC_width_in_um)) #normal?
```

Histogram of log(HC_depth_combined$HC_width_in_um)
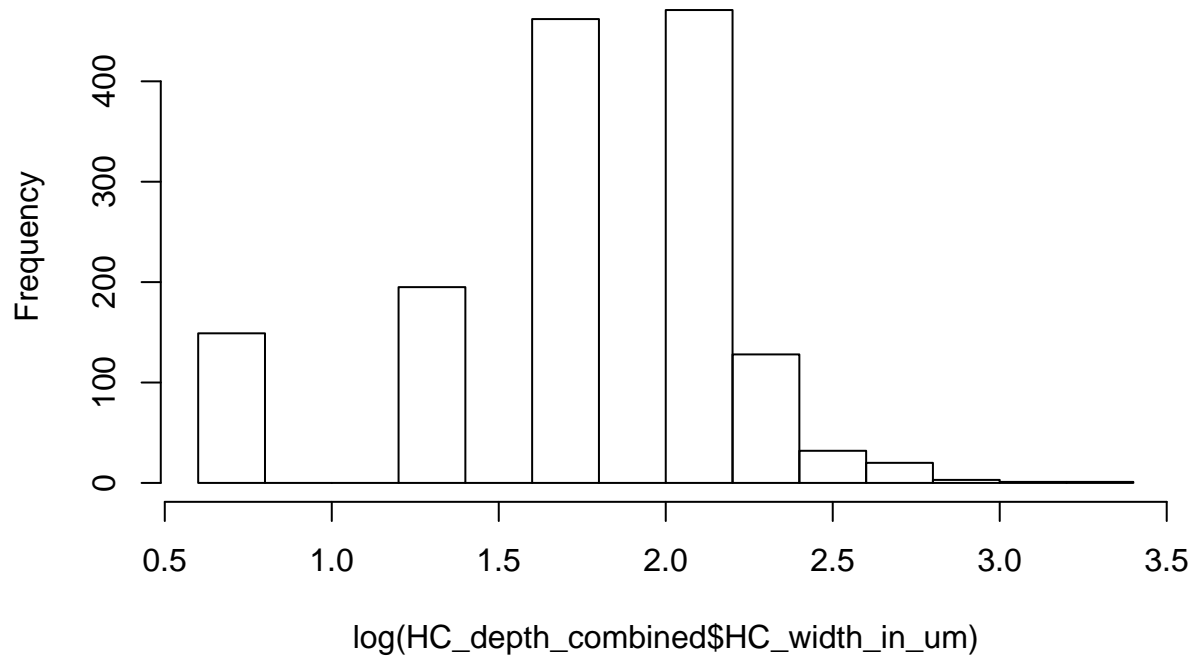

```
shapiro.test(log(HC_depth_combined$HC_width_in_um))
```

```
##
##  Shapiro-Wilk normality test
##
## data:  log(HC_depth_combined$HC_width_in_um)
## W = NaN, p-value = NA
```

```
hist(HC_cold_younger$HC_width_in_um)
```

Histogram of HC_cold_younger$HC_width_in_um

HC_cold_younger$HC_width_in_um
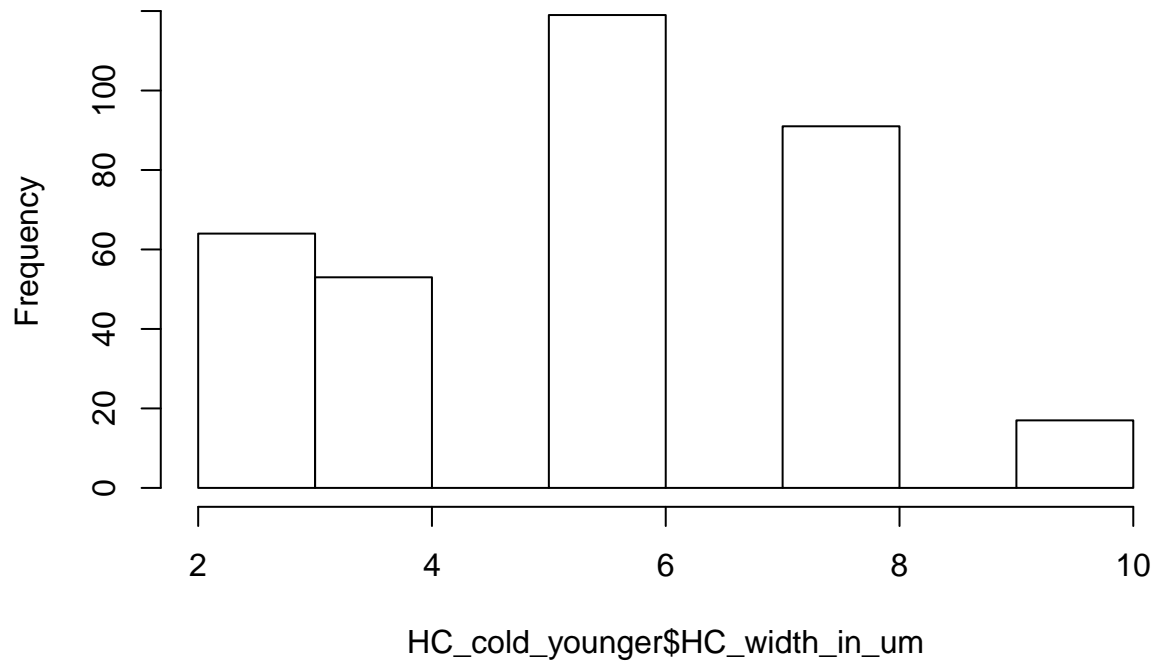
```
hist(HC_cold_older$HC_width_in_um)
```

Histogram of HC_cold_older$HC_width_in_um

HC_cold_older$HC_width_in_um

```
var.test(HC_cold_younger$HC_width_in_um, HC_cold_older$HC_width_in_um) # equal variances
```

```
##
```

```
##  F test to compare two variances
##
## data:  HC_cold_younger$HC_width_in_um and HC_cold_older$HC_width_in_um
## F = 0.65718, num df = 343, denom df = 473, p-value = 3.833e-05
## alternative hypothesis: true ratio of variances is not equal to 1
## 95 percent confidence interval:
##  0.5405910 0.8014236
## sample estimates:
## ratio of variances
##           0.6571828
```

```r
t.test(HC_cold_younger$HC_width_in_um, HC_cold_older$HC_width_in_um, alternative=c("two.sided"), paired=
```

```
##
##  Two Sample t-test
##
## data:  HC_cold_younger$HC_width_in_um and HC_cold_older$HC_width_in_um
## t = -5.7353, df = 816, p-value = 1.371e-08
## alternative hypothesis: true difference in means is not equal to 0
## 95 percent confidence interval:
##  -1.4394484 -0.7053852
## sample estimates:
## mean of x mean of y
##  5.674419  6.746835
```

Significant difference between cold-younger and cold-older.

```r
hist(HC_hot_younger$HC_width_in_um)
```

Histogram of HC_hot_younger$HC_width_in_um


```r
hist(HC_hot_older$HC_width_in_um)
```
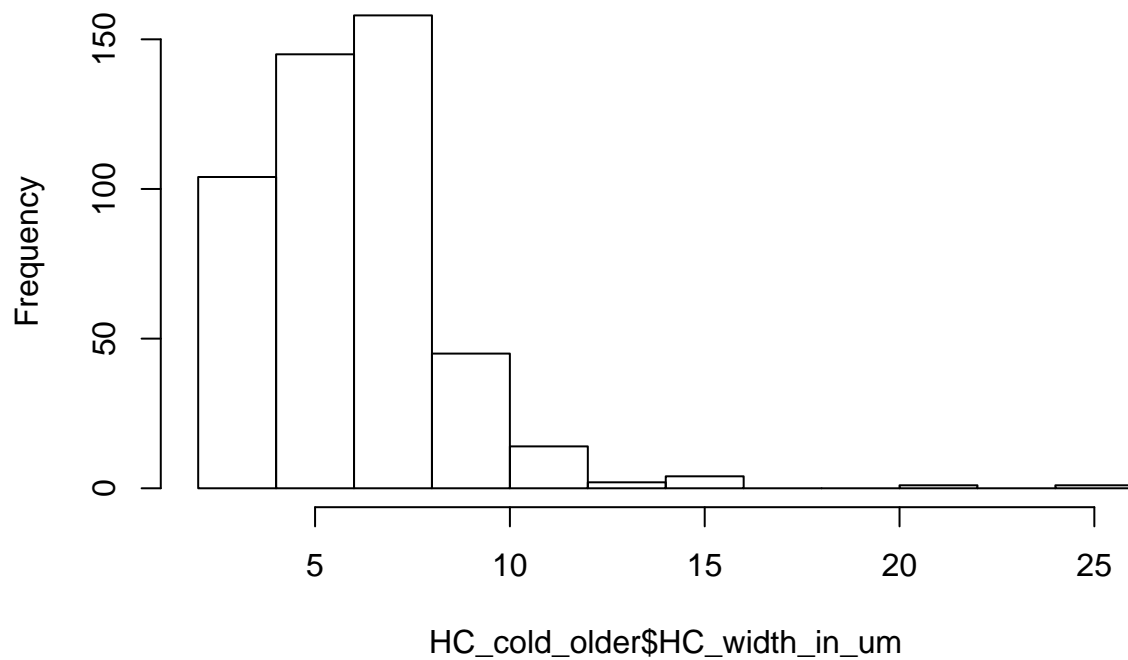
Histogram of HC_hot_older$HC_width_in_um
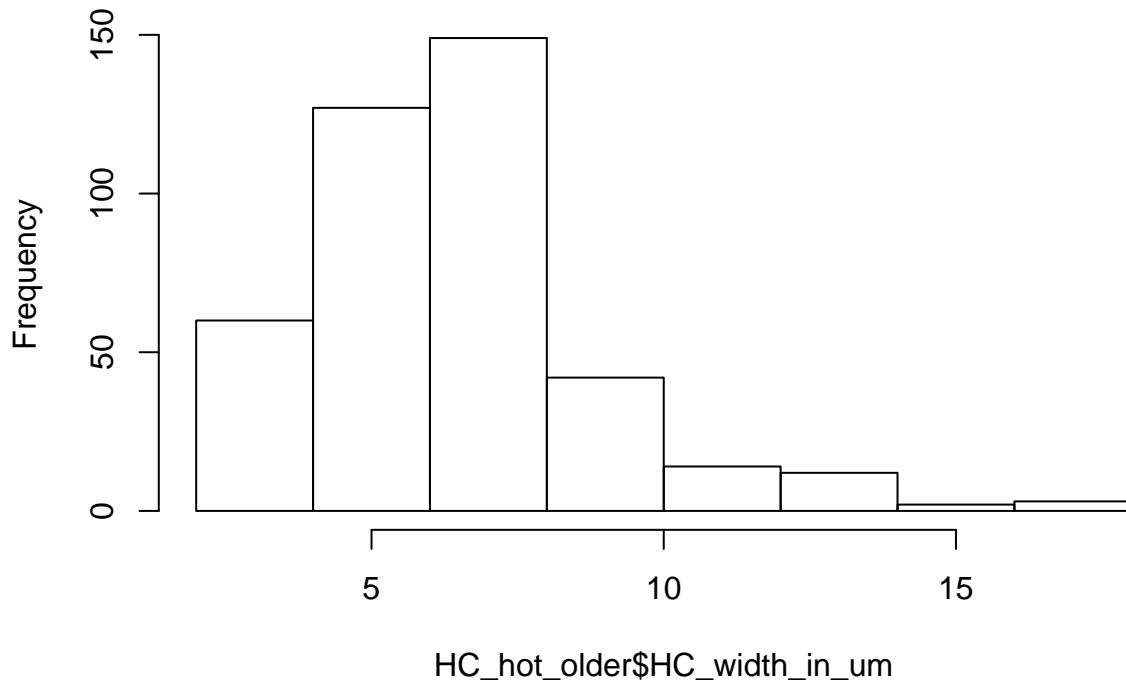
HC_hot_older$HC_width_in_um

```r
var.test(HC_hot_younger$HC_width_in_um, HC_hot_older$HC_width_in_um) # equal variances
```
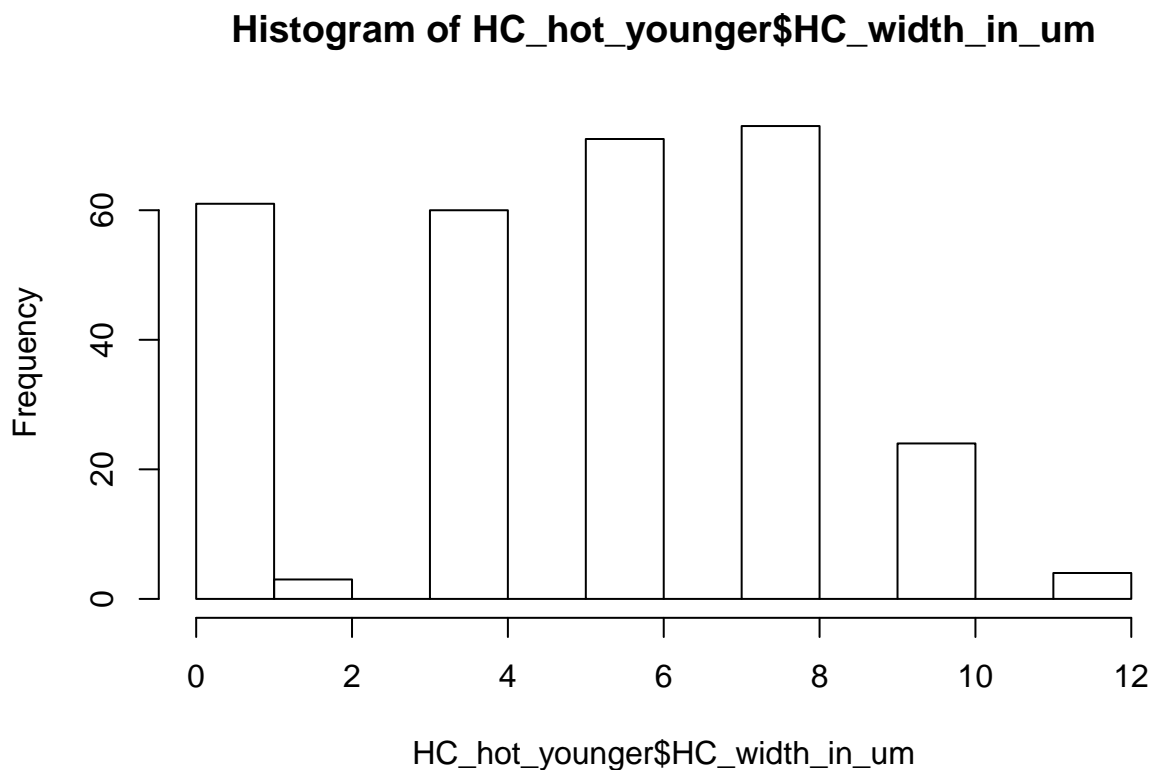
```
##
##  F test to compare two variances
##
## data:  HC_hot_younger$HC_width_in_um and HC_hot_older$HC_width_in_um
## F = 1.4137, num df = 295, denom df = 408, p-value = 0.001242
## alternative hypothesis: true ratio of variances is not equal to 1
## 95 percent confidence interval:
##   1.145544 1.751147
## sample estimates:
## ratio of variances
##           1.413745
```

```r
t.test(HC_hot_younger$HC_width_in_um, HC_hot_older$HC_width_in_um, alternative=c("two.sided"), paired=F
```

```
##
##  Two Sample t-test
##
## data:  HC_hot_younger$HC_width_in_um and HC_hot_older$HC_width_in_um
## t = -9.1724, df = 703, p-value < 2.2e-16
## alternative hypothesis: true difference in means is not equal to 0
## 95 percent confidence interval:
##  -2.518835 -1.630640
## sample estimates:
## mean of x mean of y
##  5.216216  7.290954
```

Significant difference between hot-younger and hot-older.

Significant differences between younger in hot and cold?

```r
var.test(HC_hot_younger$HC_width_in_um, HC_cold_younger$HC_width_in_um) #equal variances
```

```
##
##  F test to compare two variances
##
## data:  HC_hot_younger$HC_width_in_um and HC_cold_younger$HC_width_in_um
## F = 1.9777, num df = 295, denom df = 343, p-value = 1.325e-09
## alternative hypothesis: true ratio of variances is not equal to 1
## 95 percent confidence interval:
##  1.587842 2.467898
## sample estimates:
## ratio of variances
##           1.977721
```

```r
t.test(HC_hot_younger$HC_width_in_um, HC_cold_younger$HC_width_in_um, alternative=c("two.sided"), paired
```

```
##
##  Two Sample t-test
##
## data:  HC_hot_younger$HC_width_in_um and HC_cold_younger$HC_width_in_um
## t = -2.0733, df = 638, p-value = 0.03854
## alternative hypothesis: true difference in means is not equal to 0
## 95 percent confidence interval:
##  -0.89217999 -0.02422478
## sample estimates:
## mean of x mean of y
##  5.216216  5.674419
```

Significant difference between hot-younger and cold-younger.

```r
var.test(HC_hot_older$HC_width_in_um, HC_cold_older$HC_width_in_um) #unequal variances
```

```
##
##  F test to compare two variances
##
## data:  HC_hot_older$HC_width_in_um and HC_cold_older$HC_width_in_um
## F = 0.91935, num df = 408, denom df = 473, p-value = 0.3809
## alternative hypothesis: true ratio of variances is not equal to 1
## 95 percent confidence interval:
##  0.762649 1.109702
## sample estimates:
## ratio of variances
##          0.9193483
```

```r
t.test(HC_hot_older$HC_width_in_um, HC_cold_older$HC_width_in_um, alternative=c("two.sided"), paired=FAL
```

```
##
##  Welch Two Sample t-test
##
## data:  HC_hot_older$HC_width_in_um and HC_cold_older$HC_width_in_um
## t = 2.8886, df = 871.26, p-value = 0.003966
## alternative hypothesis: true difference in means is not equal to 0
## 95 percent confidence interval:
##  0.1744077 0.9138285
```

```
## sample estimates:
## mean of x mean of y
##  7.290954  6.746835
```

Significant difference between hot-older and cold-older.

Part III : Compare SYN depths/widths between younger and older

Read in SYN data & summarize in a table

Next, we want to read in the relevant datafile.

```
SYN_cold_younger<-read.xlsx(file="widthSYNcombined.xlsx", sheetName="ColdYounger")
SYN_cold_older<-read.xlsx(file="widthSYNcombined.xlsx", sheetName="ColdOlder")
SYN_hot_younger<-read.xlsx(file="widthSYNcombined.xlsx", sheetName="HotYounger")
SYN_hot_older<-read.xlsx(file="widthSYNcombined.xlsx", sheetName="HotOlder")
```

Don't include the Puncta results - i.e. only include columns A-E

```
SYN_cold_younger<-SYN_cold_younger[,1:5]
SYN_cold_older<-SYN_cold_older[,1:5]
SYN_hot_younger<-SYN_hot_younger[,1:5]
SYN_hot_older<-SYN_hot_older[,1:5]
```

Add a column for Treatment at the end of each dataframe

```
SYN_cold_younger$Temperature<-"Cold"
SYN_cold_older$Temperature<-"Cold"
SYN_hot_younger$Temperature<-"Hot"
SYN_hot_older$Temperature<-"Hot"

SYN_cold_younger$AgeGroup<-"Younger"
SYN_cold_older$AgeGroup<-"Older"
SYN_hot_younger$AgeGroup<-"Younger"
SYN_hot_older$AgeGroup<-"Older"
```

Let's combine the dataframes into 1 comprehensive one!

```
SYN_depth_combined<- rbind(SYN_cold_younger, SYN_cold_older, SYN_hot_younger, SYN_hot_older)
```

```
SYN_depth_summary <-
  SYN_depth_combined %>%
  group_by(Temperature, AgeGroup) %>%
  summarize(count = n(),
            mean = mean(SYN_width_in_um, na.rm=TRUE),
            SD = sd(SYN_width_in_um, na.rm=TRUE),
            SE = sd(SYN_width_in_um, na.rm=TRUE)/sqrt(n())
            )
kable(SYN_depth_summary,title="Mean & SD & SE", digits=3)
```

Temperature	AgeGroup	count	mean	SD	SE
Cold	Older	446	4.614	2.281	0.108
Cold	Younger	287	4.272	2.833	0.167
Hot	Older	408	5.961	2.519	0.125
Hot	Younger	315	4.216	2.653	0.149

```
mean(SYN_depth_summary$mean) #why we measured synapses every 4um
```

```
## [1] 4.765696
```

Plotting SYN widths/depths

Make a plot for the hot and cold temps:

```
Hot_Cold_SYN_depth_summary<-SYN_depth_summary[1:4,]
Hot_Cold_SYN_depth_summary$AgeGroup <- ordered(Hot_Cold_SYN_depth_summary$AgeGroup, levels=c("Younger",
Cold_SYN_depth_summary<-SYN_depth_summary[1:2,]
Cold_SYN_depth_summary$AgeGroup <- ordered(Cold_SYN_depth_summary$AgeGroup, levels=c("Younger", "Older")
Hot_SYN_depth_summary<-SYN_depth_summary[3:4,]
Hot_SYN_depth_summary$AgeGroup <- ordered(Hot_SYN_depth_summary$AgeGroup, levels=c("Younger", "Older"))

ggplot(data=Hot_Cold_SYN_depth_summary, aes(x=AgeGroup, y=mean), color="Temperature") +
  geom_point(data=Cold_SYN_depth_summary, size=3, color="blue") +
  geom_errorbar(data=Cold_SYN_depth_summary, aes(ymin=mean-SE, ymax=mean+SE), color="blue", width=0.05)
  geom_point(data=Hot_SYN_depth_summary, size=3, color="red") +
  geom_errorbar(data=Hot_SYN_depth_summary, aes(ymin=mean-SE, ymax=mean+SE), color="red", width=0.05)+
  theme_bw(20)+
        theme(panel.grid.major = element_blank(),
        panel.grid.minor = element_blank())+
  ylab("Synapse Width (µm)\n")+
  xlab("\n Age Group")
```
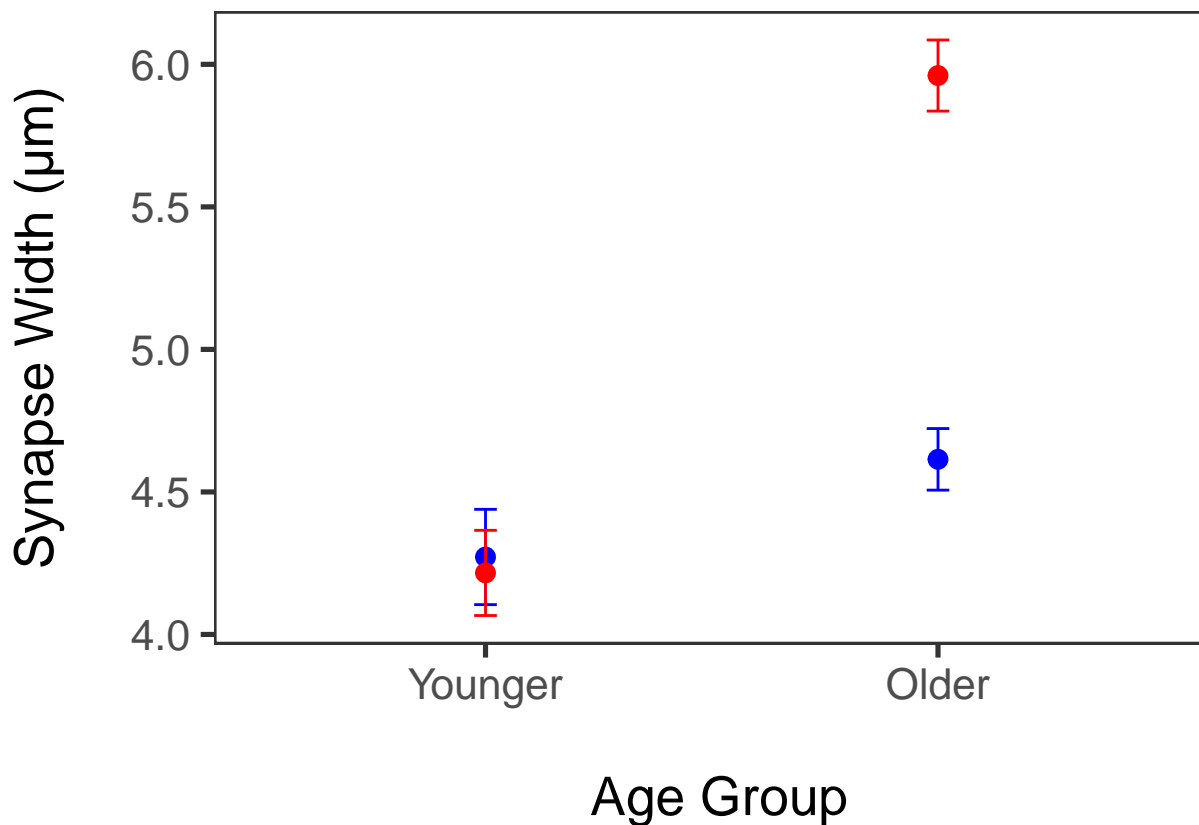
Stats for significant differences between younger and older (in hot and cold dataset)

```r
hist(SYN_depth_combined$SYN_width_in_um) #normal
```

Histogram of SYN_depth_combined$SYN_width_in_um

SYN_depth_combined$SYN_width_in_um
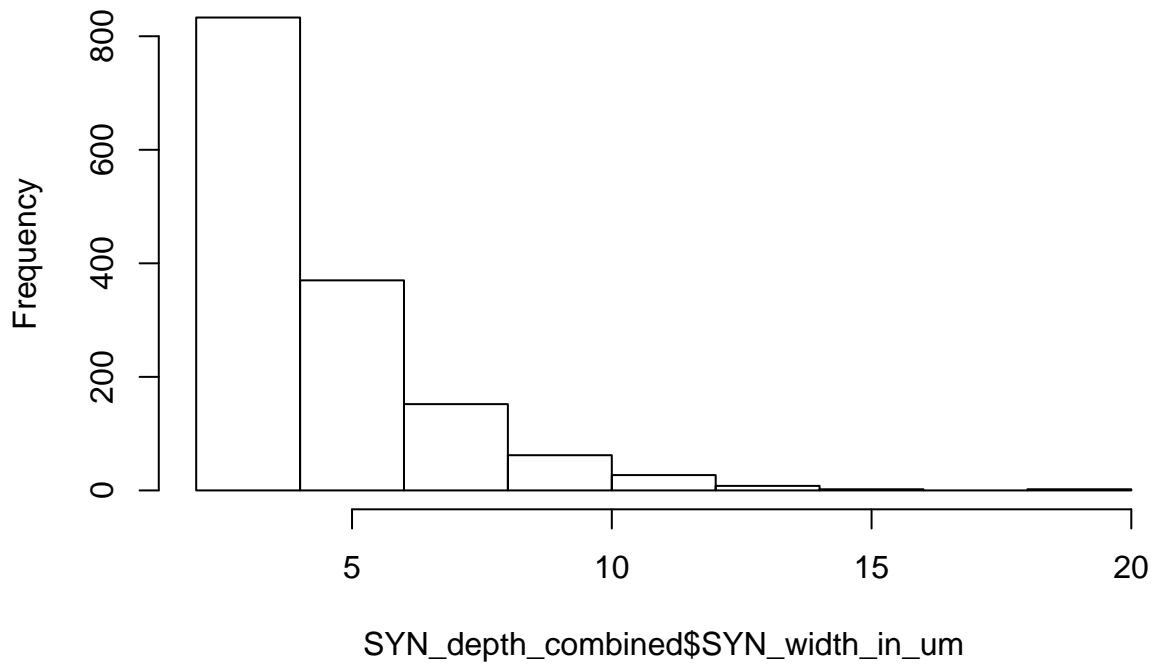
```r
hist(SYN_cold_younger$SYN_width_in_um)
```

Histogram of SYN_cold_younger$SYN_width_in_um

SYN_cold_younger$SYN_width_in_um
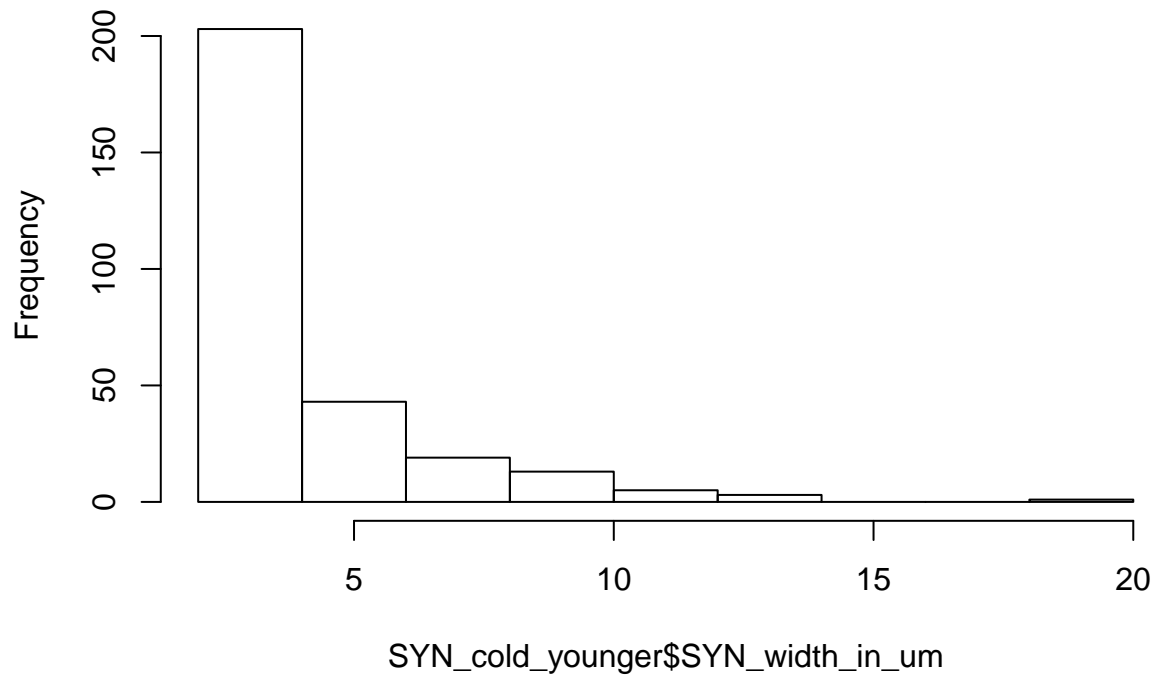
```
hist(SYN_cold_older$SYN_width_in_um)
```

Histogram of SYN_cold_older$SYN_width_in_um

SYN_cold_older$SYN_width_in_um
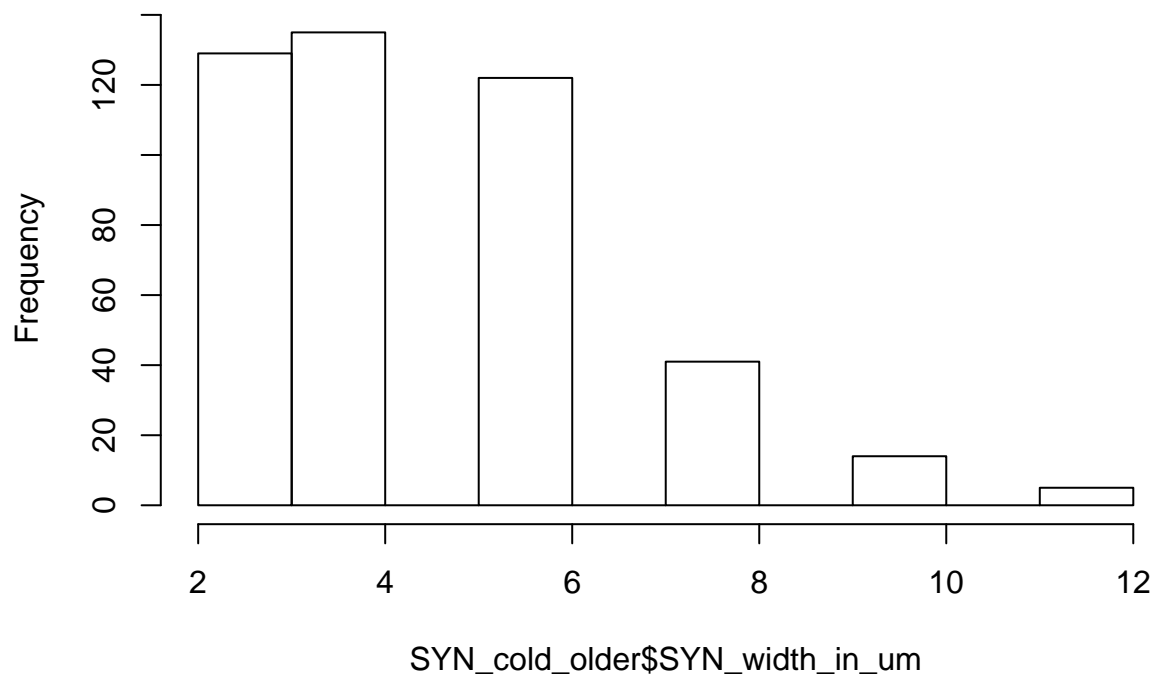
```
var.test(SYN_cold_younger$SYN_width_in_um, SYN_cold_older$SYN_width_in_um) # equal variances
```

```
##
```

```
##  F test to compare two variances
##
## data:  SYN_cold_younger$SYN_width_in_um and SYN_cold_older$SYN_width_in_um
## F = 1.5419, num df = 286, denom df = 445, p-value = 4.244e-05
## alternative hypothesis: true ratio of variances is not equal to 1
## 95 percent confidence interval:
##  1.252381 1.907691
## sample estimates:
## ratio of variances
##           1.541923
```

```
t.test(SYN_cold_younger$SYN_width_in_um, SYN_cold_older$SYN_width_in_um, alternative=c("two.sided"), pa
```

```
##
##  Two Sample t-test
##
## data:  SYN_cold_younger$SYN_width_in_um and SYN_cold_older$SYN_width_in_um
## t = -1.8026, df = 731, p-value = 0.07187
## alternative hypothesis: true difference in means is not equal to 0
## 95 percent confidence interval:
##  -0.71567278  0.03052723
## sample estimates:
## mean of x mean of y
##  4.271777  4.614350
```

No significant difference between cold-younger and cold-older.

```
hist(SYN_hot_younger$SYN_width_in_um)
```


Histogram of SYN_hot_younger$SYN_width_in_um

```
hist(SYN_hot_older$SYN_width_in_um)
```

Histogram of SYN_hot_older$SYN_width_in_um

SYN_hot_older$SYN_width_in_um
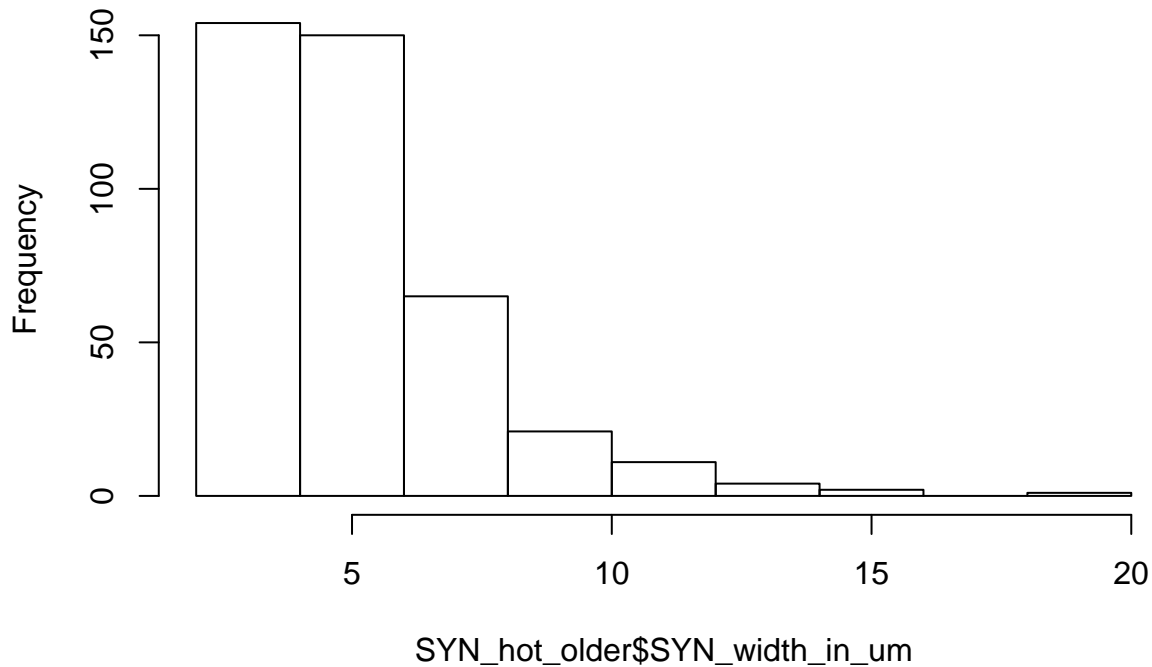
```r
var.test(SYN_hot_younger$SYN_width_in_um, SYN_hot_older$SYN_width_in_um) # unequal
```

```
## 
##  F test to compare two variances
## 
## data:  SYN_hot_younger$SYN_width_in_um and SYN_hot_older$SYN_width_in_um
## F = 1.1085, num df = 314, denom df = 407, p-value = 0.3293
## alternative hypothesis: true ratio of variances is not equal to 1
## 95 percent confidence interval:
##  0.9011324 1.3674721
## sample estimates:
## ratio of variances
##            1.108502
```

```r
t.test(SYN_hot_younger$SYN_width_in_um, SYN_hot_older$SYN_width_in_um, alternative=c("two.sided"), paire
```

```
## 
##  Welch Two Sample t-test
## 
## data:  SYN_hot_younger$SYN_width_in_um and SYN_hot_older$SYN_width_in_um
## t = -8.9637, df = 657.61, p-value < 2.2e-16
## alternative hypothesis: true difference in means is not equal to 0
## 95 percent confidence interval:
##  -2.127148 -1.362674
## sample estimates:
## mean of x mean of y
##  4.215873  5.960784
```

Significant difference between hot-younger and hot-older.

Significant differences between younger in hot and cold?

```
var.test(SYN_hot_younger$SYN_width_in_um, SYN_cold_younger$SYN_width_in_um) #unequal variances
```
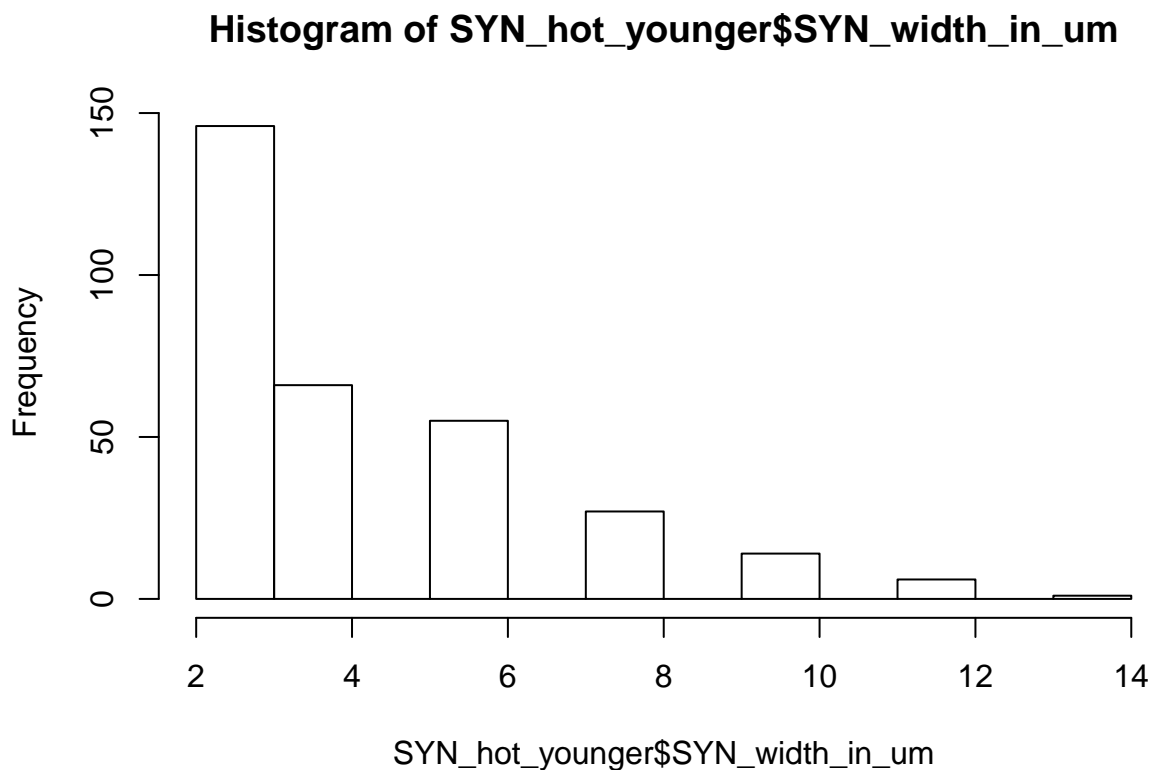
```
##
##  F test to compare two variances
##
## data:  SYN_hot_younger$SYN_width_in_um and SYN_cold_younger$SYN_width_in_um
## F = 0.8769, num df = 314, denom df = 286, p-value = 0.2551
## alternative hypothesis: true ratio of variances is not equal to 1
## 95 percent confidence interval:
##  0.6983965 1.0996866
## sample estimates:
## ratio of variances
##           0.8768999
```

```
t.test(SYN_hot_younger$SYN_width_in_um, SYN_cold_younger$SYN_width_in_um, alternative=c("two.sided"), pa
```

```
##
##  Welch Two Sample t-test
##
## data:  SYN_hot_younger$SYN_width_in_um and SYN_cold_younger$SYN_width_in_um
## t = -0.24928, df = 585.28, p-value = 0.8032
## alternative hypothesis: true difference in means is not equal to 0
## 95 percent confidence interval:
##  -0.4963631  0.3845552
## sample estimates:
## mean of x mean of y
##  4.215873  4.271777
```

NO significant difference between hot-younger and cold-younger.

```
var.test(SYN_hot_older$SYN_width_in_um, SYN_cold_older$SYN_width_in_um) #barely equal variances
```

```
##
##  F test to compare two variances
##
## data:  SYN_hot_older$SYN_width_in_um and SYN_cold_older$SYN_width_in_um
## F = 1.2198, num df = 407, denom df = 445, p-value = 0.0404
## alternative hypothesis: true ratio of variances is not equal to 1
## 95 percent confidence interval:
##  1.008740 1.476145
## sample estimates:
## ratio of variances
##           1.219765
```

```
t.test(SYN_hot_older$SYN_width_in_um, SYN_cold_older$SYN_width_in_um, alternative=c("two.sided"), paired
```

```
##
##  Two Sample t-test
##
## data:  SYN_hot_older$SYN_width_in_um and SYN_cold_older$SYN_width_in_um
## t = 8.1963, df = 852, p-value = 9.061e-16
## alternative hypothesis: true difference in means is not equal to 0
## 95 percent confidence interval:
##  1.024007 1.668862
```

```
## sample estimates:
## mean of x mean of y
##  5.960784  4.614350
```

YES significant difference between hot-older and cold-older.

Part IV : Number of Hair Cells

N of hair cells per scan

Here we'll calculate the AVERAGE TOTAL number of hair cells per SCAN per age per treatment (so total # HCs in scan/surface NOT avg per slice per scan).

```r
Hot_total <- Hot[!is.na(Hot$avg_total_N_hair_cells),]
# total_hot_summary <-
#   Hot_total %>%
#   group_by(HatchingState) %>%
#   summarize(count = n(),
#             mean = mean(avg_total_N_hair_cells, na.rm=TRUE),
#             SD = sd(avg_total_N_hair_cells, na.rm=TRUE),
#             SE = sd(avg_total_N_hair_cells, na.rm=TRUE)/sqrt(n())
#             )
# total_hot_summary$Temperature <- "Hot"
# kable(total_hot_summary,title="Mean & SD & SE", digits=3)
```

```r
Cold_total <- Cold[!is.na(Cold$avg_total_N_hair_cells),]
# total_cold_summary <-
#   Cold_total %>%
#   group_by(HatchingState) %>%
#   summarize(count = n(),
#             mean = mean(avg_total_N_hair_cells, na.rm=TRUE),
#             SD = sd(avg_total_N_hair_cells, na.rm=TRUE),
#             SE = sd(avg_total_N_hair_cells, na.rm=TRUE)/sqrt(n())
#             )
# total_cold_summary$Temperature <- "Cold"
# kable(total_cold_summary,title="Mean & SD & SE", digits=3)
```

N of hair cells per ear

Here we'll calculate the AVERAGE number of hair cells per EAR per age per treatment (so we'll sum up the total # HCs in each scan in each ear).

First combine the Individual and ear columns to create an individual identifier for each ear.

```r
totals_combined <- rbind(Cold_total, Hot_total)
```

The above includes the total HC count per SCAN.

```r
totals_combined$ear <- paste(totals_combined$Individual,totals_combined$Which_ear)
```

Create a table summary of statistics that combines multiple scans for each ear.

```r
# total_HC_by_ear_summary <-
#   totals_combined %>%
#   group_by(ear) %>%
#   summarize(count = n(),
```

```
#           mean = mean(Kaylee_Total_N_of_hair_cells, na.rm=TRUE),
#           SD = sd(Kaylee_Total_N_of_hair_cells, na.rm=TRUE),
#           SE = sd(Kaylee_Total_N_of_hair_cells, na.rm=TRUE)/sqrt(n())
#           )
# #total_HC_by_ear_summary$Temperature <- "Cold"
# kable(total_HC_by_ear_summary,title="Mean & SD & SE", digits=3)
```

I want to split this table by temperature treatment (and age group within that).

These are the cold treated ears:

```
Cold_total$ear <- paste(Cold_total$Individual,Cold_total$Which_ear)
cold_HC_by_ear_summary <-
  Cold_total %>%
  group_by(HatchingState, ear, Clutch, Stage) %>% #show these variables
  summarize(count = n(),
            mean = mean(avg_total_N_hair_cells, na.rm=TRUE),
            SD = sd(avg_total_N_hair_cells, na.rm=TRUE),
            SE = sd(avg_total_N_hair_cells, na.rm=TRUE)/sqrt(n())
            )
cold_HC_by_ear_summary$Temperature <- "Cold"
kable(cold_HC_by_ear_summary,title="Mean & SD & SE", digits=3)
```

HatchingState	ear	Clutch	Stage	count	mean	SD	SE	Temperature
0	120 left	406	28	2	29.000	18.385	13.000	Cold
0	120 right	406	28	1	44.000	NA	NA	Cold
0	132 left	412	28	4	13.000	3.464	1.732	Cold
0	132 right	412	28	4	31.625	23.725	11.863	Cold
0	35 right	313	27	1	11.000	NA	NA	Cold
0	63 left	318	27	3	13.667	8.520	4.919	Cold
0	77 left	372	27	4	20.500	16.708	8.354	Cold
0	77 right	372	27	2	25.750	23.688	16.750	Cold
0	78 right	373	27	3	42.833	2.021	1.167	Cold
1	129 left	406	28	2	40.500	21.213	15.000	Cold
1	129 right	406	28	4	25.125	12.392	6.196	Cold
1	133 right	412	28	5	16.900	7.241	3.238	Cold
1	135 left	410	28	2	49.500	7.778	5.500	Cold
1	135 right	410	28	3	15.667	7.522	4.343	Cold
1	136 right	412	28	2	31.000	26.163	18.500	Cold
1	51 right	314	28	3	7.000	4.000	2.309	Cold
1	55 left	313	28	1	50.000	NA	NA	Cold
1	55 right	313	28	2	27.500	21.213	15.000	Cold
1	66 left	318	29	4	16.875	4.768	2.384	Cold
1	82 left	372	28	3	46.500	28.649	16.540	Cold
1	82 right	372	28	3	33.000	37.693	21.762	Cold
1	84 left	373	28	3	18.500	17.197	9.929	Cold
1	87 right	374	29	3	31.833	9.713	5.608	Cold

These are the hot treated ears:

```
Hot_total$ear <- paste(Hot_total$Individual,Hot_total$Which_ear)
hot_HC_by_ear_summary <-
  Hot_total %>%
  group_by(HatchingState, ear, Clutch, Stage) %>% #show these variables
```

```
    summarize(count = n(),
          mean = mean(avg_total_N_hair_cells, na.rm=TRUE),
          SD = sd(avg_total_N_hair_cells, na.rm=TRUE),
          SE = sd(avg_total_N_hair_cells, na.rm=TRUE)/sqrt(n())
          )
hot_HC_by_ear_summary$Temperature <- "Hot"
kable(hot_HC_by_ear_summary,title="Mean & SD & SE", digits=3)
```

HatchingState	ear	Clutch	Stage	count	mean	SD	SE	Temperature
0	147 left	428	27	5	20.000	19.206	8.589	Hot
0	148 left	416	27	3	12.333	6.714	3.877	Hot
0	148 right	416	27	3	23.000	15.025	8.675	Hot
0	150 left	421	27	4	34.875	30.486	15.243	Hot
0	4 left	292	27	1	32.500	NA	NA	Hot
0	4 right	292	27	1	31.000	NA	NA	Hot
1	158 left	420	29	5	28.700	20.444	9.143	Hot
1	158 right	420	29	3	30.833	24.871	14.359	Hot
1	159 left	421	27	1	27.500	NA	NA	Hot
1	162 left	428	29	2	23.750	3.889	2.750	Hot
1	162 right	428	29	3	41.667	15.495	8.946	Hot
1	163 left	416	28	1	37.500	NA	NA	Hot
1	163 right	416	28	2	16.750	8.839	6.250	Hot
1	5 left	291	28	4	39.625	22.765	11.382	Hot
1	6 left	292	29	2	28.500	7.071	5.000	Hot
1	6 right	292	29	4	21.875	10.680	5.340	Hot
1	7 right	291	28	2	16.000	3.536	2.500	Hot

Let's combine the hot/cold tables into 1 comprehensive one!

```
hotcold_HC_by_ear<- rbind(hot_HC_by_ear_summary, cold_HC_by_ear_summary)
kable(hotcold_HC_by_ear,title="Mean & SD & SE", digits=3)
```

HatchingState	ear	Clutch	Stage	count	mean	SD	SE	Temperature
0	147 left	428	27	5	20.000	19.206	8.589	Hot
0	148 left	416	27	3	12.333	6.714	3.877	Hot
0	148 right	416	27	3	23.000	15.025	8.675	Hot
0	150 left	421	27	4	34.875	30.486	15.243	Hot
0	4 left	292	27	1	32.500	NA	NA	Hot
0	4 right	292	27	1	31.000	NA	NA	Hot
1	158 left	420	29	5	28.700	20.444	9.143	Hot
1	158 right	420	29	3	30.833	24.871	14.359	Hot
1	159 left	421	27	1	27.500	NA	NA	Hot
1	162 left	428	29	2	23.750	3.889	2.750	Hot
1	162 right	428	29	3	41.667	15.495	8.946	Hot
1	163 left	416	28	1	37.500	NA	NA	Hot
1	163 right	416	28	2	16.750	8.839	6.250	Hot
1	5 left	291	28	4	39.625	22.765	11.382	Hot
1	6 left	292	29	2	28.500	7.071	5.000	Hot
1	6 right	292	29	4	21.875	10.680	5.340	Hot
1	7 right	291	28	2	16.000	3.536	2.500	Hot
0	120 left	406	28	2	29.000	18.385	13.000	Cold
0	120 right	406	28	1	44.000	NA	NA	Cold

HatchingState	ear	Clutch	Stage	count	mean	SD	SE	Temperature
0	132 left	412	28	4	13.000	3.464	1.732	Cold
0	132 right	412	28	4	31.625	23.725	11.863	Cold
0	35 right	313	27	1	11.000	NA	NA	Cold
0	63 left	318	27	3	13.667	8.520	4.919	Cold
0	77 left	372	27	4	20.500	16.708	8.354	Cold
0	77 right	372	27	2	25.750	23.688	16.750	Cold
0	78 right	373	27	3	42.833	2.021	1.167	Cold
1	129 left	406	28	2	40.500	21.213	15.000	Cold
1	129 right	406	28	4	25.125	12.392	6.196	Cold
1	133 right	412	28	5	16.900	7.241	3.238	Cold
1	135 left	410	28	2	49.500	7.778	5.500	Cold
1	135 right	410	28	3	15.667	7.522	4.343	Cold
1	136 right	412	28	2	31.000	26.163	18.500	Cold
1	51 right	314	28	3	7.000	4.000	2.309	Cold
1	55 left	313	28	1	50.000	NA	NA	Cold
1	55 right	313	28	2	27.500	21.213	15.000	Cold
1	66 left	318	29	4	16.875	4.768	2.384	Cold
1	82 left	372	28	3	46.500	28.649	16.540	Cold
1	82 right	372	28	3	33.000	37.693	21.762	Cold
1	84 left	373	28	3	18.500	17.197	9.929	Cold
1	87 right	374	29	3	31.833	9.713	5.608	Cold

```
hotcold_HC_by_ear$totalHCperEar <- hotcold_HC_by_ear$mean
hotcold_by_ear_summary <-
  hotcold_HC_by_ear %>%
  group_by(Temperature, HatchingState) %>% #show these variables
  summarize(count = n(),
            mean = mean(totalHCperEar),
            SD = sd(totalHCperEar),
            SE = sd(totalHCperEar)/sqrt(n())
            )
```

prints table

```
kable(hotcold_by_ear_summary,title="Mean & SD & SE", digits=3)
```

| Temperature	HatchingState	count	mean	SD	SE
Cold	0	9	25.708	12.368	4.123
Cold	1	14	29.279	13.614	3.638
Hot	0	6	25.618	8.682	3.545
Hot	1	11	28.427	8.633	2.603

Plotting number of HCs per ear

The following code creates our figure:

```
ggplot(data=hotcold_by_ear_summary, aes(x=HatchingState, y=mean, colour=Temperature)) +
  theme_bw(20)+
        theme(panel.grid.major = element_blank(),
        panel.grid.minor = element_blank())+
```

```
geom_point(size=3) +
scale_colour_manual(values = c("blue", "red")) +
theme(legend.position="none") +
geom_errorbar(data=hotcold_by_ear_summary, aes(ymin=mean-SE, ymax=mean+SE), width=0.05)+
ylab("# Hair Cells Per Ear \n")+
xlab("\n Age Group")
```
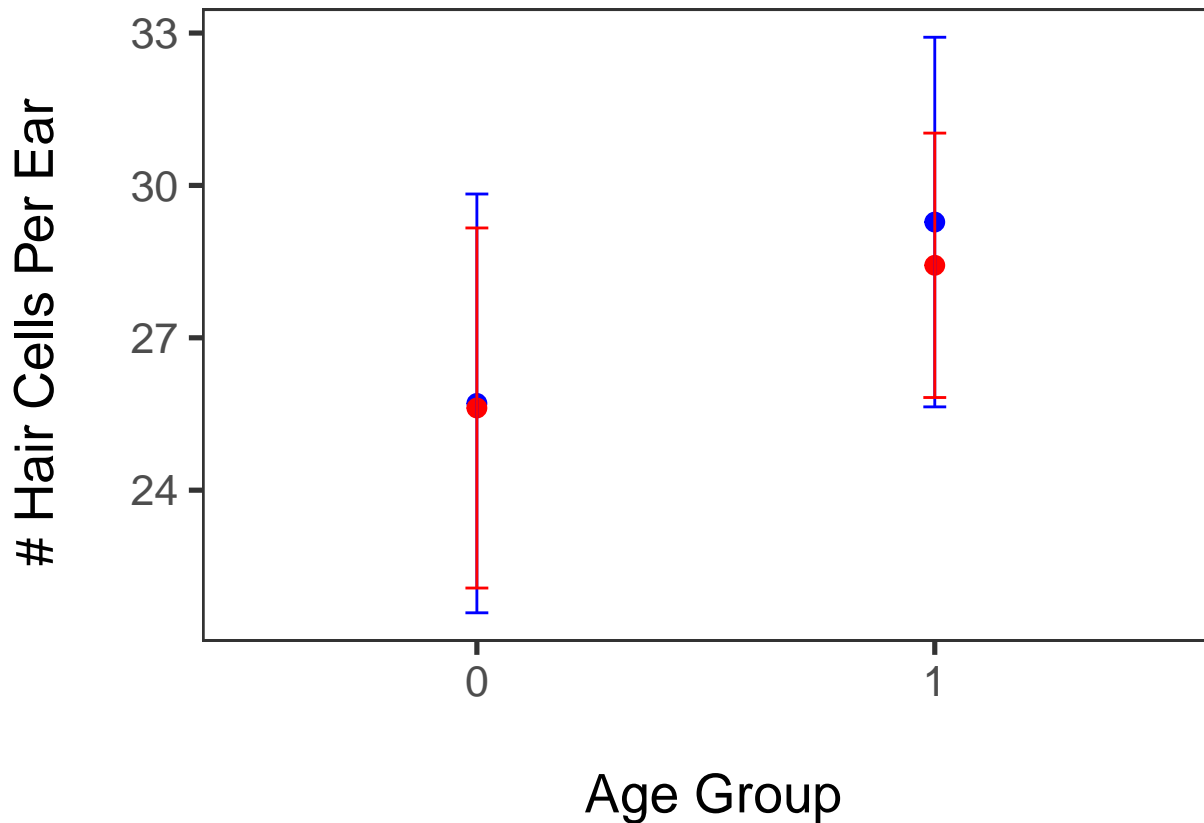

Plotting number of HCs per ear

Stats for HC abundance by temps:

```
hot_HC_by_ear<-subset(hotcold_HC_by_ear, Temperature=="Hot")
cold_HC_by_ear<-subset(hotcold_HC_by_ear, Temperature=="Cold")

noH_hot_HC_by_ear<-subset(hot_HC_by_ear, HatchingState==0)
yesH_hot_HC_by_ear<-subset(hot_HC_by_ear, HatchingState==1)

t.test(noH_hot_HC_by_ear$totalHCperEar, yesH_hot_HC_by_ear$totalHCperEar, alternative=c("two.sided"), pa
```

```
##
##  Welch Two Sample t-test
##
## data:  noH_hot_HC_by_ear$totalHCperEar and yesH_hot_HC_by_ear$totalHCperEar
## t = -0.6388, df = 10.343, p-value = 0.5368
## alternative hypothesis: true difference in means is not equal to 0
## 95 percent confidence interval:
```

```
## -12.563980    6.945545
## sample estimates:
## mean of x mean of y
##  25.61806  28.42727
```

```
noH_cold_HC_by_ear<-subset(cold_HC_by_ear, HatchingState==0)
YesH_cold_HC_by_ear<-subset(cold_HC_by_ear, HatchingState==1)

t.test(noH_cold_HC_by_ear$totalHCperEar, YesH_cold_HC_by_ear$totalHCperEar, alternative=c("two.sided"),
```

```
##
##  Welch Two Sample t-test
##
## data:  noH_cold_HC_by_ear$totalHCperEar and YesH_cold_HC_by_ear$totalHCperEar
## t = -0.64931, df = 18.434, p-value = 0.5241
## alternative hypothesis: true difference in means is not equal to 0
## 95 percent confidence interval:
##  -15.102742    7.962266
## sample estimates:
## mean of x mean of y
##  25.70833  29.27857
```

What is the average stage for younger and older treatments?

```
stages_summary <-
  hotcold_HC_by_ear %>%
  group_by(HatchingState) %>% #show these variables
  summarize(count = n(),
            mode = Mode(Stage),
            mean = mean(Stage),
            SD = sd(Stage),
            SE = sd(Stage)/sqrt(n())
            )
kable(stages_summary,title="Mean & SD & SE", digits=3)
```

HatchingState	count	mode	mean	SD	SE
0	15	27	27.267	0.458	0.118
1	25	28	28.280	0.542	0.108

The modal stage for younger group is 27 and the modal stage for the older group is 28.

Part V: HC Surface Area

First we want to get a value per scan of the HC surface area.

Summarize the data for

Here we'll create a table summary of statistics for HC surface area for each age category, grouped by stimulus treatment. We use the {dplyr} package to summarize our data.

```
hot_HCtHC_summary <-
  Hot %>%
  group_by(HatchingState) %>%
  summarize(count = n(),
            mean = mean(Average_HC_to_HC_surface_area, na.rm=TRUE),
            SD = sd(Average_HC_to_HC_surface_area, na.rm=TRUE),
            SE = sd(Average_HC_to_HC_surface_area, na.rm=TRUE)/sqrt(n())
            )
hot_HCtHC_summary$Temperature <- "Hot"
kable(hot_HCtHC_summary,title="Mean & SD & SE", digits=3)
```

HatchingState	count	mean	SD	SE	Temperature
0	254	1133.347	1129.989	70.902	Hot
1	506	1255.433	1133.053	50.370	Hot

```
cold_HCtHC_summary <-
  Cold %>%
  group_by(HatchingState) %>%
  summarize(count = n(),
            mean = mean(Average_HC_to_HC_surface_area, na.rm=TRUE),
            SD = sd(Average_HC_to_HC_surface_area, na.rm=TRUE),
            SE = sd(Average_HC_to_HC_surface_area, na.rm=TRUE)/sqrt(n())
            )
cold_HCtHC_summary$Temperature <- "Cold"
kable(cold_HCtHC_summary,title="Mean & SD & SE", digits=3)
```

HatchingState	count	mean	SD	SE	Temperature
0	391	1086.279	1106.950	55.981	Cold
1	636	1443.251	1358.003	53.848	Cold

Let's combine the hot and cold tables into 1 comprehensive one!

```
hotcold_HCtHC_summary<- rbind(cold_HCtHC_summary, hot_HCtHC_summary)
```

**relevels Younger –> Medium –> Older**

```
#hotcold_HCtHC_summary$HatchingState <- ordered(hotcold_HCtHC_summary$HatchingState, levels=c("Younger"
```

prints table

The following code creates our figure:

```
ggplot(data=hotcold_HCtHC_summary, aes(x=HatchingState, y=mean, colour=Temperature)) +
  theme_bw(20)+
    theme(panel.grid.major = element_blank(),
          panel.grid.minor = element_blank())+
  geom_point(size=3) +
  scale_colour_manual(values = c("blue", "red")) +
  theme(legend.position="none") +
```

```
geom_errorbar(data=hotcold_HCtHC_summary, aes(ymin=mean-SE, ymax=mean+SE), width=0.05)+
ylab("HC Surface Area (um^2)\n")+
xlab("\n Age Group")
```
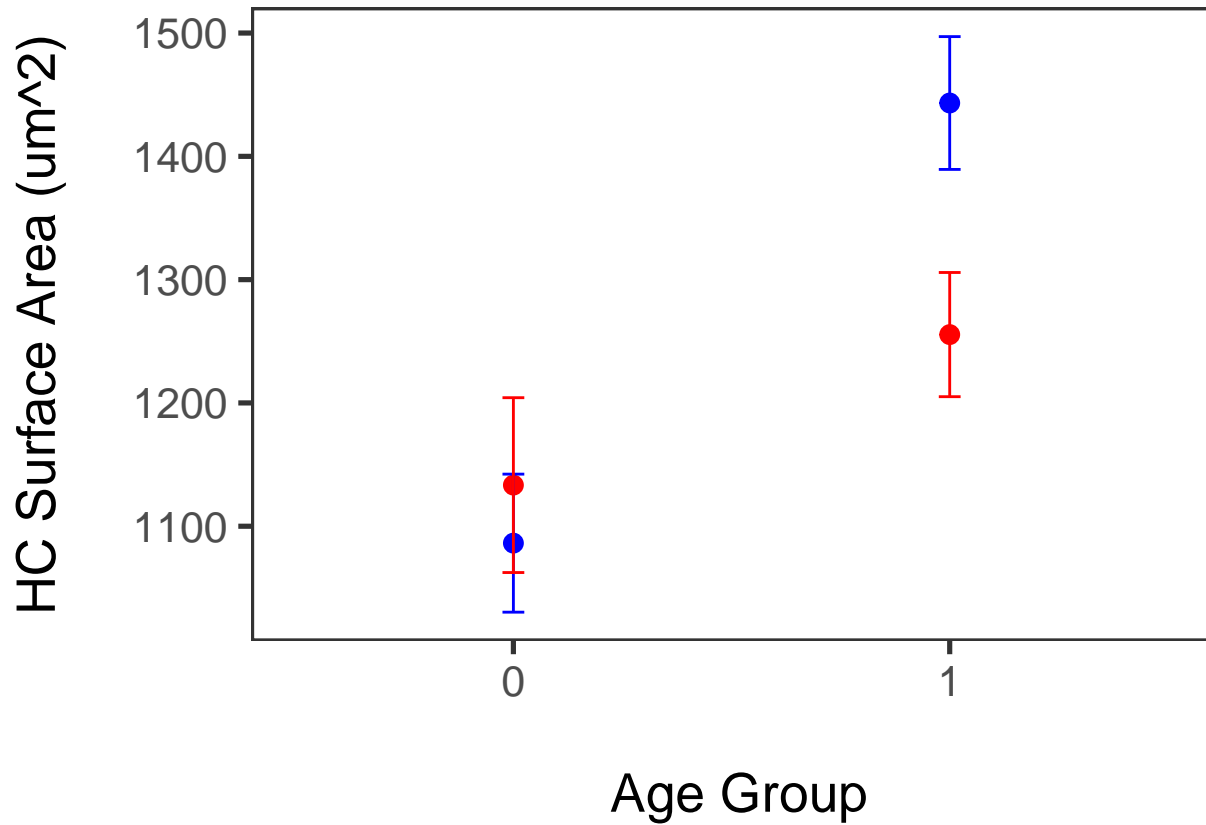

Summarize the data for

Here we'll create a table summary of statistics for HC surface area for each age category, grouped by stimulus treatment. We use the {dplyr} package to summarize our data.

```
hot_HC_SA_summary <-
  Hot %>%
  group_by(HatchingState) %>%
  summarize(count = n(),
            mean = mean(Average_HC_surface_area, na.rm=TRUE),
            SD = sd(Average_HC_surface_area, na.rm=TRUE),
            SE = sd(Average_HC_surface_area, na.rm=TRUE)/sqrt(n())
            )
hot_HC_SA_summary$Temperature <- "Hot"
kable(hot_HC_SA_summary,title="Mean & SD & SE", digits=3)
```

HatchingState	count	mean	SD	SE	Temperature
0	254	2459.288	1893.127	118.785	Hot
1	506	2616.268	1892.543	84.134	Hot

```
cold_HC_SA_summary <-
  Cold %>%
  group_by(HatchingState) %>%
  summarize(count = n(),
            mean = mean(Average_HC_surface_area, na.rm=TRUE),
            SD = sd(Average_HC_surface_area, na.rm=TRUE),
            SE = sd(Average_HC_surface_area, na.rm=TRUE)/sqrt(n())
            )
cold_HC_SA_summary$Temperature <- "Cold"
kable(cold_HC_SA_summary,title="Mean & SD & SE", digits=3)
```

HatchingState	count	mean	SD	SE	Temperature
0	391	2166.838	1735.021	87.744	Cold
1	636	3061.943	2469.588	97.926	Cold

Let's combine the tables into 1 comprehensive one for hot/cold data!

```
hotcold_HC_SA_summary<- rbind(cold_HC_SA_summary, hot_HC_SA_summary)
kable(hotcold_HC_SA_summary,title="Mean & SD & SE", digits=3)
```

HatchingState	count	mean	SD	SE	Temperature
0	391	2166.838	1735.021	87.744	Cold
1	636	3061.943	2469.588	97.926	Cold
0	254	2459.288	1893.127	118.785	Hot
1	506	2616.268	1892.543	84.134	Hot

relevels Younger –> Medium –> Older

```
#hotcold_HC_SA_summary$HatchingState <- ordered(hotcold_HC_SA_summary$HatchingState, levels=c("Younger"
```

The following code creates our figure:

```
ggplot(data=hotcold_HC_SA_summary, aes(x=HatchingState, y=mean, colour=Temperature)) +
  geom_point(size=3) +
    theme_bw(20)+
  scale_colour_manual(values = c("blue", "red")) +
  theme(legend.position="none") +
  geom_errorbar(data=hotcold_HC_SA_summary, aes(ymin=mean-SE, ymax=mean+SE), width=0.05)+
    theme(panel.grid.major = element_blank(),
          panel.grid.minor = element_blank())+
  ylab("HC Surface Area (um^2)\n")+
  xlab("\n Age Group")
```
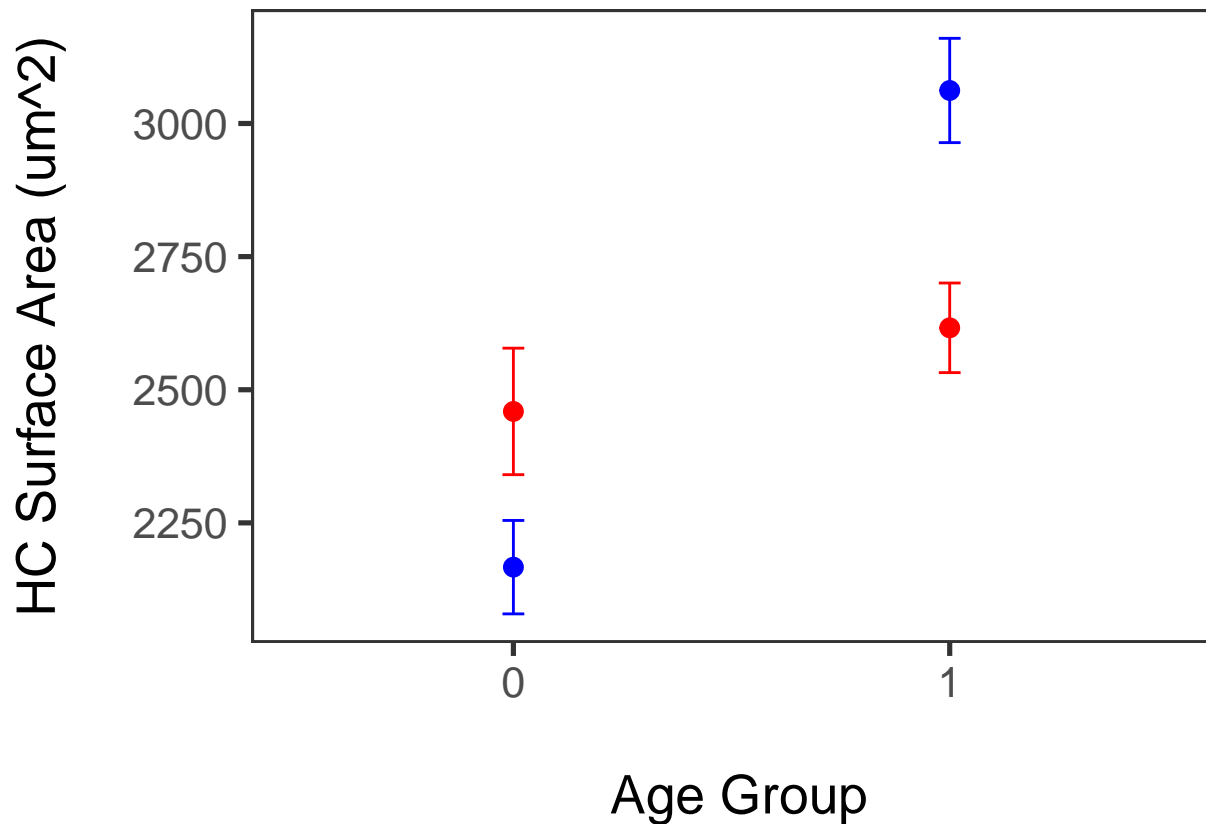
Stats for HC surface area by temps:

```
# hot_HC_by_ear<-subset(hotcold_HC_by_ear, Temperature=="Hot")
# cold_HC_by_ear<-subset(hotcold_HC_by_ear, Temperature=="Cold")
#
# noH_hot_HC_by_ear<-subset(hot_HC_by_ear, HatchingState=="Younger")
# yesH_hot_HC_by_ear<-subset(hot_HC_by_ear, HatchingState=="Older")
#
# t.test(noH_hot_HC_by_ear$totalHCperEar, yesH_hot_HC_by_ear$totalHCperEar, alternative=c("two.sided"),
#
# noH_cold_HC_by_ear<-subset(cold_HC_by_ear, HatchingState=="Younger")
# YesH_cold_HC_by_ear<-subset(cold_HC_by_ear, HatchingState=="Older")
#
# t.test(noH_cold_HC_by_ear$totalHCperEar, YesH_cold_HC_by_ear$totalHCperEar, alternative=c("two.sided"),
```

Part VI: Number of Synapses

N of synapses per scan

Here we'll calculate the AVERAGE number of synapses per SCAN per age per treatment (so total # synapses in scan NOT avg per slice per scan).

```
Hot_SYN_total <- Hot[!is.na(Hot$average_N_synapses),]

total_hot_SYN_summary <-
```

```
  Hot_SYN_total %>%
  group_by(HatchingState) %>%
  summarize(count = n(),
            mean = mean(average_N_synapses, na.rm=TRUE),
            SD = sd(average_N_synapses, na.rm=TRUE),
            SE = sd(average_N_synapses, na.rm=TRUE)/sqrt(n())
            )
total_hot_SYN_summary$Temperature <- "Hot"
kable(total_hot_SYN_summary,title="Mean & SD & SE", digits=3)
```

HatchingState	count	mean	SD	SE	Temperature
0	136	5.971	4.919	0.422	Hot
1	279	5.345	4.591	0.275	Hot

```
Cold_SYN_total <- Cold[!is.na(Cold$average_N_synapses),]

total_cold_SYN_summary <-
  Cold_SYN_total %>%
  group_by(HatchingState) %>%
  summarize(count = n(),
            mean = mean(average_N_synapses, na.rm=TRUE),
            SD = sd(average_N_synapses, na.rm=TRUE),
            SE = sd(average_N_synapses, na.rm=TRUE)/sqrt(n())
            )
total_cold_SYN_summary$Temperature <- "Cold"
kable(total_cold_SYN_summary,title="Mean & SD & SE", digits=3)
```

HatchingState	count	mean	SD	SE	Temperature
0	207	3.205	3.011	0.209	Cold
1	327	4.956	4.376	0.242	Cold

Let's combine the tables into 1 comprehensive one for hot/cold temps!

```
hotcold_SYN_by_ear<- rbind(total_hot_SYN_summary, total_cold_SYN_summary)
kable(hotcold_SYN_by_ear,title="Mean & SD & SE", digits=3)
```

HatchingState	count	mean	SD	SE	Temperature
0	136	5.971	4.919	0.422	Hot
1	279	5.345	4.591	0.275	Hot
0	207	3.205	3.011	0.209	Cold
1	327	4.956	4.376	0.242	Cold

The following code creates our figure:

```
ggplot(data=hotcold_SYN_by_ear, aes(x=HatchingState, y=mean, colour=Temperature)) +
  theme_bw(20)+
    theme(panel.grid.major = element_blank(),
          panel.grid.minor = element_blank())+
  geom_point(size=3) +
```

```
  scale_colour_manual(values = c("blue", "red")) +
  theme(legend.position="none") +
  geom_errorbar(data=hotcold_SYN_by_ear, aes(ymin=mean-SE, ymax=mean+SE), width=0.05)+
  ylab("Number of Synapses")+
  xlab("Age Group")
```
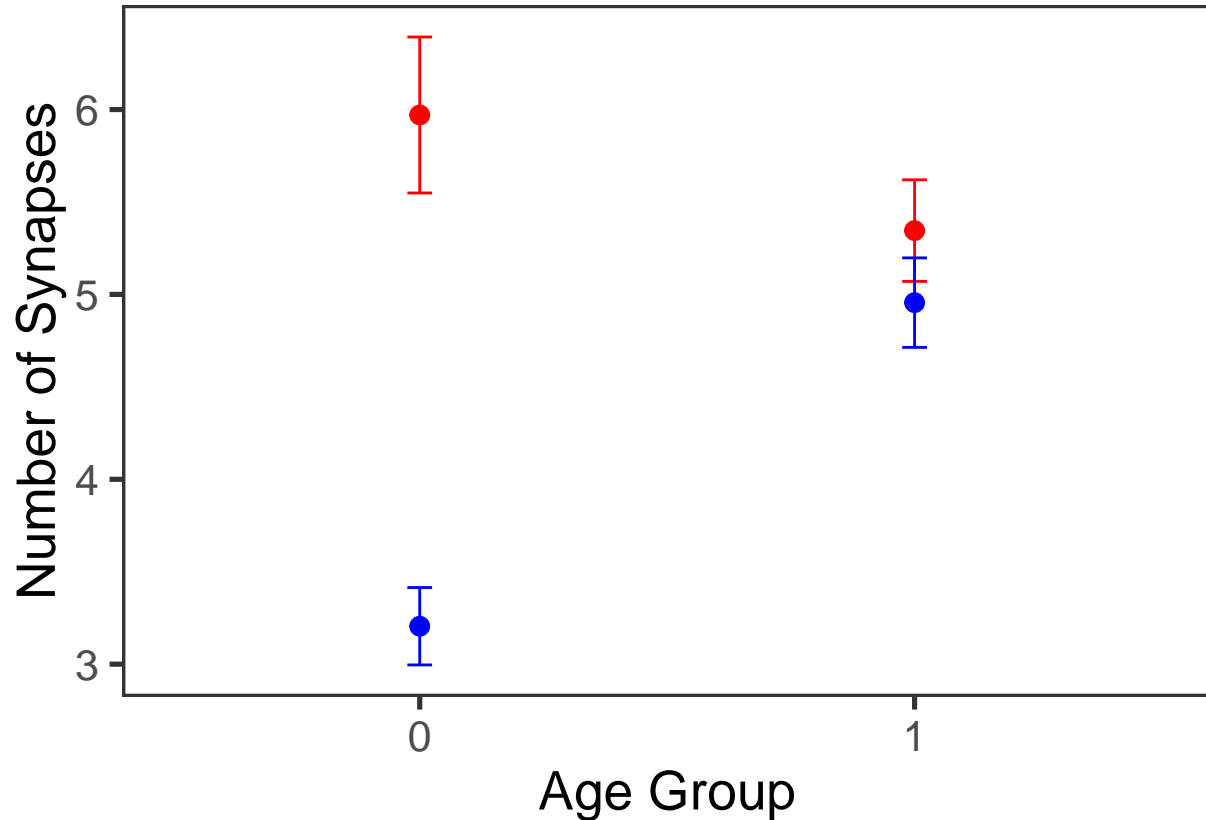

Stats for SYN abundance by temps:

```
noH_hot_SYN_by_ear<-subset(Hot_SYN_total, HatchingState==0)
yesH_hot_SYN_by_ear<-subset(Hot_SYN_total, HatchingState==1)

t.test(noH_hot_SYN_by_ear$average_N_synapses, yesH_hot_SYN_by_ear$average_N_synapses, alternative=c("two
```

```
##
##  Welch Two Sample t-test
##
## data:  noH_hot_SYN_by_ear$average_N_synapses and yesH_hot_SYN_by_ear$average_N_synapses
## t = 1.242, df = 251.9, p-value = 0.2154
## alternative hypothesis: true difference in means is not equal to 0
## 95 percent confidence interval:
##  -0.3662154  1.6168304
## sample estimates:
## mean of x mean of y
##  5.970588  5.345281
```

```
noH_cold_SYN_by_ear<-subset(Cold_SYN_total, HatchingState==0)
yesH_cold_SYN_by_ear<-subset(Cold_SYN_total, HatchingState==1)

t.test(noH_cold_SYN_by_ear$average_N_synapses, yesH_cold_SYN_by_ear$average_N_synapses, alternative=c("

##
##  Welch Two Sample t-test
##
## data:  noH_cold_SYN_by_ear$average_N_synapses and yesH_cold_SYN_by_ear$average_N_synapses
## t = -5.4708, df = 528.32, p-value = 6.928e-08
## alternative hypothesis: true difference in means is not equal to 0
## 95 percent confidence interval:
##  -2.378864 -1.121823
## sample estimates:
## mean of x mean of y
##  3.205314  4.955657
```

Part VII: Number of Synapses via SiCE

N of synapses per scan via SiCE

Here we'll calculate the AVERAGE number of synapses per SCAN per age per treatment (so total # synapses in scan NOT avg per slice per scan).

```
SiCE_Hot_SYN_total <- Hot[!is.na(Hot$Kaylee_SiCE_N_synapses),]
SiCE_total_hot_SYN_summary <-
  SiCE_Hot_SYN_total %>%
  group_by(HatchingState) %>%
  summarize(count = n(),
            mean = mean(Kaylee_SiCE_N_synapses, na.rm=TRUE),
            SD = sd(Kaylee_SiCE_N_synapses, na.rm=TRUE),
            SE = sd(Kaylee_SiCE_N_synapses, na.rm=TRUE)/sqrt(n())
            )
SiCE_total_hot_SYN_summary$Temperature <- "Hot"
kable(SiCE_total_hot_SYN_summary,title="Mean & SD & SE", digits=3)
```

HatchingState	count	mean	SD	SE	Temperature
0	236	8.072	18.128	1.180	Hot
1	491	13.458	30.736	1.387	Hot

```
SiCE_Cold_SYN_total <- Cold[!is.na(Cold$Kaylee_SiCE_N_synapses),]
SiCE_total_cold_SYN_summary <-
  SiCE_Cold_SYN_total %>%
  group_by(HatchingState) %>%
  summarize(count = n(),
            mean = mean(Kaylee_SiCE_N_synapses, na.rm=TRUE),
            SD = sd(Kaylee_SiCE_N_synapses, na.rm=TRUE),
            SE = sd(Kaylee_SiCE_N_synapses, na.rm=TRUE)/sqrt(n())
            )
SiCE_total_cold_SYN_summary$Temperature <- "Cold"
kable(SiCE_total_cold_SYN_summary,title="Mean & SD & SE", digits=3)
```

| HatchingState	count	mean	SD	SE	Temperature
0	384	12.518	25.919	1.323	Cold
1	606	15.926	31.771	1.291	Cold

Let's combine the tables into 1 comprehensive one for hot/cold temps!

```
SiCE_hotcold_SYN_by_ear<- rbind(SiCE_total_hot_SYN_summary, SiCE_total_cold_SYN_summary)
kable(SiCE_hotcold_SYN_by_ear,title="Mean & SD & SE", digits=3)
```

HatchingState	count	mean	SD	SE	Temperature
0	236	8.072	18.128	1.180	Hot
1	491	13.458	30.736	1.387	Hot
0	384	12.518	25.919	1.323	Cold
1	606	15.926	31.771	1.291	Cold

The following code creates our figure:

```
ggplot(data=SiCE_hotcold_SYN_by_ear, aes(x=HatchingState, y=mean, colour=Temperature)) +
  theme_bw(20)+
    theme(panel.grid.major = element_blank(),
          panel.grid.minor = element_blank())+
  geom_point(size=3) +
  scale_colour_manual(values = c("blue", "red")) +
  theme(legend.position="none") +
  geom_errorbar(data=SiCE_hotcold_SYN_by_ear, aes(ymin=mean-SE, ymax=mean+SE), width=0.05)+
  ylab("Number of Synapses via SiCE")+
  xlab("Age Group")
```
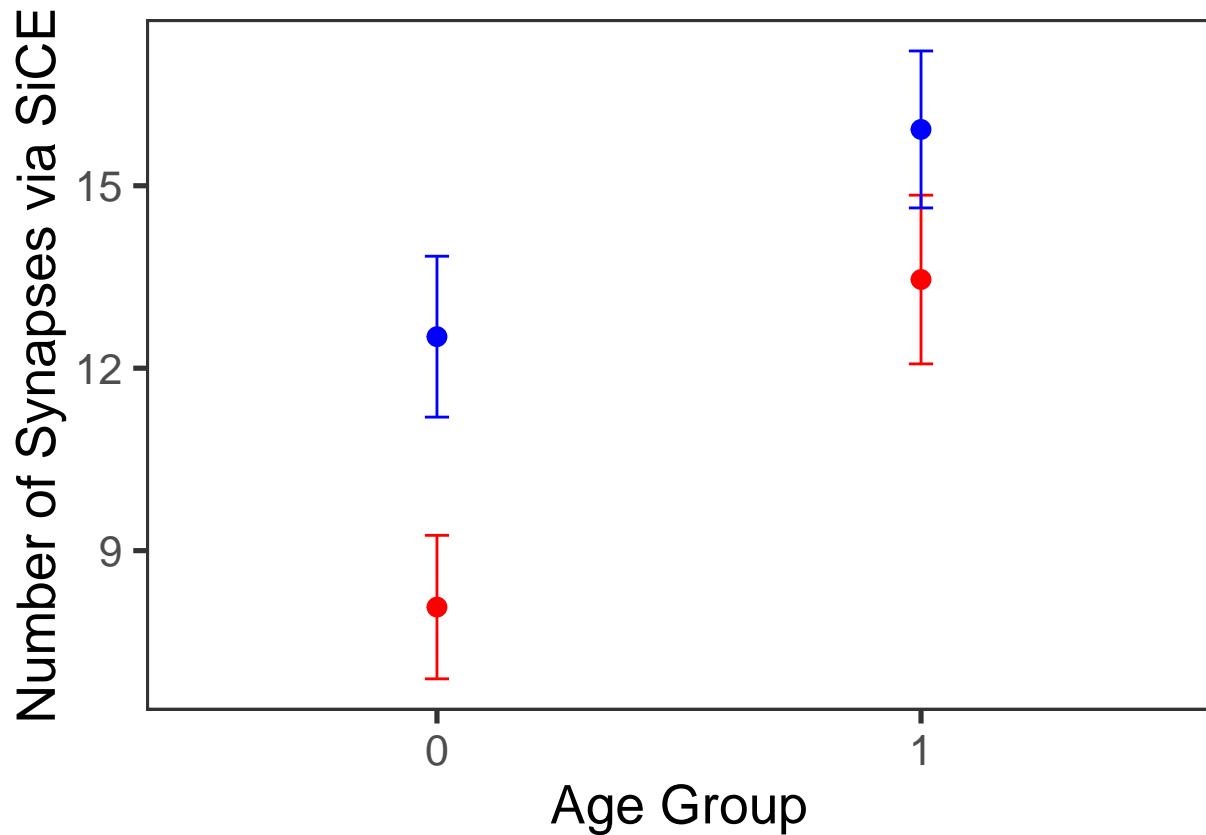
Stats for SYN abundance by temps:

```
SiCE_noH_hot_SYN_by_ear<-subset(SiCE_Hot_SYN_total, HatchingState==0)
SiCE_yesH_hot_SYN_by_ear<-subset(SiCE_Hot_SYN_total, HatchingState==1)

t.test(SiCE_noH_hot_SYN_by_ear$Kaylee_SiCE_N_synapses, SiCE_yesH_hot_SYN_by_ear$Kaylee_SiCE_N_synapses,
```

```
##
##  Welch Two Sample t-test
##
## data:  SiCE_noH_hot_SYN_by_ear$Kaylee_SiCE_N_synapses and SiCE_yesH_hot_SYN_by_ear$Kaylee_SiCE_N_syna
## t = -2.9577, df = 695.9, p-value = 0.003205
## alternative hypothesis: true difference in means is not equal to 0
## 95 percent confidence interval:
##  -8.961748 -1.810681
## sample estimates:
## mean of x mean of y
##   8.072034 13.458248
```

```
SiCE_noH_cold_SYN_by_ear<-subset(SiCE_Cold_SYN_total, HatchingState==0)
SiCE_yesH_cold_SYN_by_ear<-subset(SiCE_Cold_SYN_total, HatchingState==1)

t.test(SiCE_noH_cold_SYN_by_ear$Kaylee_SiCE_N_synapses, SiCE_yesH_cold_SYN_by_ear$Kaylee_SiCE_N_synapses
```

```
##
##  Welch Two Sample t-test
##
```

```
## data:  SiCE_noH_cold_SYN_by_ear$Kaylee_SiCE_N_synapses and SiCE_yesH_cold_SYN_by_ear$Kaylee_SiCE_N_sy
## t = -1.8439, df = 927.33, p-value = 0.06552
## alternative hypothesis: true difference in means is not equal to 0
## 95 percent confidence interval:
##  -7.0342746  0.2192478
## sample estimates:
## mean of x mean of y
##  12.51823  15.92574
```
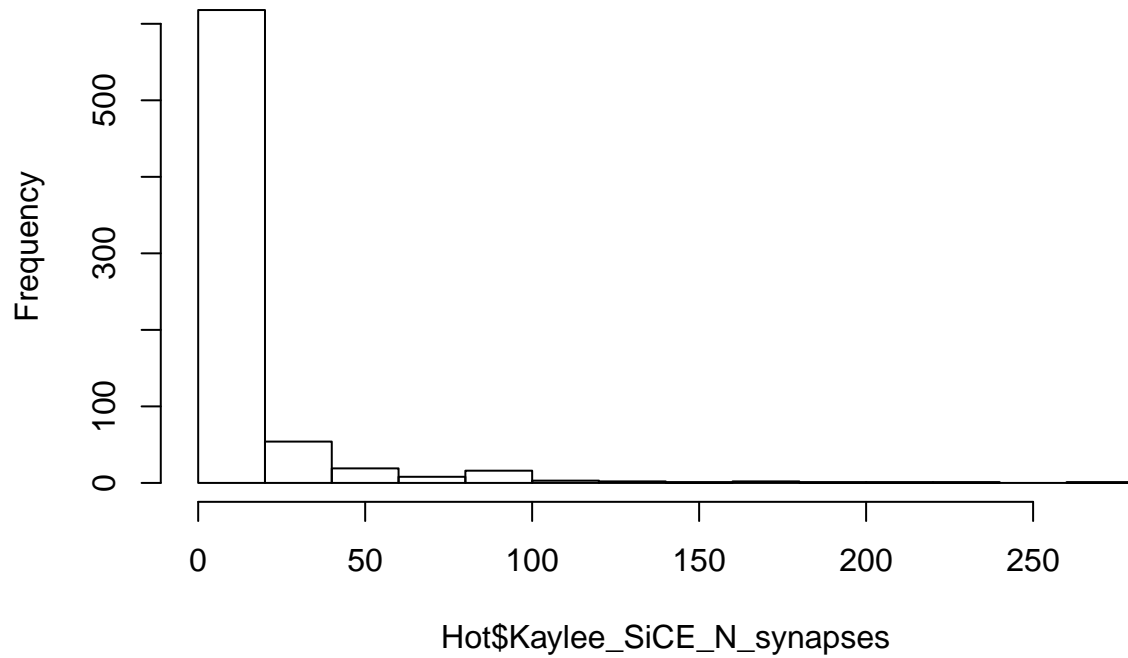
take out the 0s and the super high ones

```r
hist(data$Kaylee_SiCE_N_synapses)
```

Histogram of data$Kaylee_SiCE_N_synapses
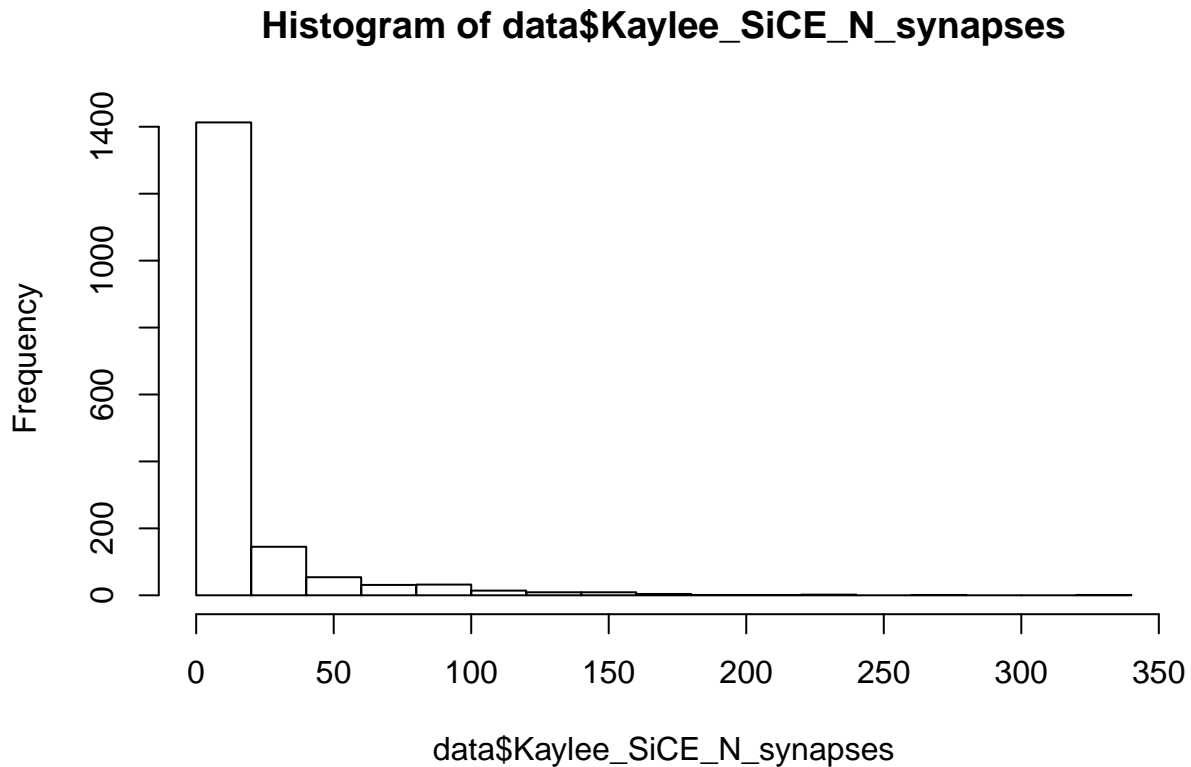

```r
hist(Hot$Kaylee_SiCE_N_synapses)
```

Histogram of Hot$Kaylee_SiCE_N_synapses


```
hist(Cold$Kaylee_SiCE_N_synapses)
```

Histogram of Cold$Kaylee_SiCE_N_synapses

Part VIII: Synapse Density