

Females responses to glareosin

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```
#data  
glareosin_data<-read.csv("glareosin_behavioural_data.csv", header=T)
```

```
#packages  
library(ggplot2)  
library(car)  
library(gridExtra)  
library(cowplot)  
library(ggsignif)
```

```
#change variables to factors  
glareosin_data$tile<-as.factor(glareosin_data$tile)  
glareosin_data$test<-as.factor(glareosin_data$test)  
  
#check all females repsonded in tests  
glareosin_data$vole_id<-as.factor(glareosin_data$vole_id)  
ggplot(data=glareosin_data, aes(x=test, y=time.sniff.tile)) + geom_point(aes(colour=vole_id)) +  
  ylim(0,60)  
ggplot(data=glareosin_data, aes(x=test, y=time.on.tile)) + geom_point(aes(colour=vole_id)) +  
  ylim(0,300)  
#looks like all females showed some response
```

```

#check distributions

#time on tile
glareosin_data$lgtime.on.tile<-log(glareosin_data$time.on.tile+1)
glareosin_data$sqrt.time.on.tile<-sqrt(glareosin_data$time.on.tile)

par(mfrow=c(3,1))
#histograms
hist(glareosin_data$time.on.tile,main="time on tile")
hist(glareosin_data$lgtime.on.tile,main="log +1")
hist(glareosin_data$sqrt.time.on.tile,main="sqrt")

#qqplots
qqPlot(glareosin_data$time.on.tile, main="time on tile")
qqPlot(glareosin_data$lgtime.on.tile,main="log +1")
qqPlot(glareosin_data$sqrt.time.on.tile, main="sqrt")

#shapiro tests
shapiro.test(glareosin_data$time.on.tile)
shapiro.test(glareosin_data$lgtime.on.tile)
shapiro.test(glareosin_data$sqrt.time.on.tile)
#log data for time on tile looks fine

#time sniffing stimulus

glareosin_data$lgsniff.tile<-log(glareosin_data$time.sniff.tile)
glareosin_data$sqrt.sniff.tile<-sqrt(glareosin_data$time.sniff.tile)

#histograms
hist(glareosin_data$time.sniff.tile, main="time sniffing")
hist(glareosin_data$lgsniff.tile, main="log +1")
hist(glareosin_data$sqrt.sniff.tile, main="sqrt")

#qqplots
qqPlot(glareosin_data$time.sniff.tile, main="time sniffing")
qqPlot(glareosin_data$lgsniff.tile, main="log +1")
qqPlot(glareosin_data$sqrt.sniff.tile, main="sqrt")

#shapiro test
shapiro.test(glareosin_data$time.sniff.tile)
shapiro.test(glareosin_data$lgsniff.tile)
shapiro.test(glareosin_data$sqrt.sniff.tile)
#square root for time sniffing tile looks fine

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```
#comparing breeding and non-breeding urine with paired t-tests
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```
#seperate out tests comparing breeding (b) and non-breeding urine (nb)
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```
glareosin_bvnb<-glareosin_data[glareosin_data$test=="nb b",]
```

```
glareosin_bvnb$test<-as.factor(glareosin_bvnb$test)
```

```
glareosin_bvnb$tile<-factor(glareosin_bvnb$tile, levels = c('b', 'nb'))
```

```
glareosin_time_bvnb.tt<-t.test(lgtime.on.tile~tile, data=glareosin_bvnb, paired=T)
```

```
glareosin_time_bvnb.tt
```

```
glareosin_sniff_bvnb.tt<-t.test(sqrt.sniff.tile~tile, data=glareosin_bvnb, paired=T)
```

```
glareosin_sniff_bvnb.tt
```

```
# comparing non-breeding urine and non-breeding urine spikedw with glareosin, paired t tests
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```
#seperate out tests comparing non breeding urine (nb) and non-breeding urine spiked with glareosin (nbg)
```

```
glareosin_nbgvnb<-glareosin_data[glareosin_data$test=="nb nbg",]
```

```
glareosin_nbgvnb$test<-as.factor(glareosin_nbgvnb$test)
```

```
glareosin_nbgvnb$tile<-factor(glareosin_nbgvnb$tile, levels = c('nbg', 'nb'))
```

```
glareosin_time_nbgvnb.tt<-t.test(lgtime.on.tile~tile, data=glareosin_nbgvnb, paired=T)
```

```
glareosin_time_nbgvnb.tt
```

```
glareosin_sniff_nbgvnb.tt<-t.test(sqrt.sniff.tile~tile, data=glareosin_nbgvnb, paired=T)
```

```
glareosin_sniff_nbgvnb.tt
```

```

#fig 4a-d
glareosin_data$test<-factor(glareosin_data$test, levels=c("nb nbg", "nb b"))
glareosin_data$tile<-factor(glareosin_data$tile, levels=c("nb","nbg","b"))

#add signifcance
sniff_sig <- data.frame(signif = c("NS", "NS"),
                        y_position = c(60,60),
                        group = c(1,2),
                        start = c("nb", "nb"),
                        end = c("nbg", "b"),
                        test =c('nb nbg','nb b'))

sniff_sig$test<-factor(sniff_sig$test, levels=c("nb nbg", "nb b"))
#time sniffing stimulus boxplot
sniff.gg<-ggplot(data=glareosin_data, aes(x=tile, y=time.sniff.tile)) +
  (geom_boxplot(aes(fill=tile), outlier.shape=NA)) +
  geom_point(position=position_jitterdodge(),aes(shape=tile),
            stroke=1.5, colour="black", size=3) +
  geom_signif(aes(
    xmin= start,
    xmax= end,
    y_position = y_position,
    annotations = signif),
    data = sniff_sig , manual = TRUE, size=1, textsize = 10, tip_length = 0) +
  facet_wrap(.~test, scales="free") + theme_bw() +
  ylab("Time sniffing \n stimulus (s)") +
  theme(axis.title.x = element_blank(),
        axis.text.x = element_blank()) +
  theme(axis.title.y =element_text(size=32, face= "bold"),
        axis.text.y = element_text(size=26),
        panel.grid.minor=element_blank(),
        panel.grid.major = element_blank(),
        axis.line = element_line(colour = "black", size=0.5),
        strip.background = element_blank(),
        panel.border = element_blank(),
        strip.text.x = element_blank()) +
  theme(legend.position = "none") +
  scale_shape_manual(values=c(17,15,19)) +
  scale_y_continuous(expand = expansion(mult = c(0.05, .1))) +
  scale_fill_manual(values=c("#7DD6D4", "#AE2AB1", '#E48100'))

sniff.gg

#add signifcance
time_sig <- data.frame(signif = c("NS", "NS"),
                      y_position = c(260,260),
                      group = c(1,2),
                      start = c("b", "nb"),
                      end = c("nb", "nbg"),
                      test =c("nb b", 'nb nbg'))

time_sig$test<-factor(time_sig$test, levels=c("nb nbg", "nb b"))

#time on tile boxplot

```

```

time.gg<-ggplot(data=glareosin_data, aes(x=tile, y=time.on.tile)) +
  (geom_boxplot(aes(fill=tile), outlier.shape=NA)) +
  geom_point(position=position_jitterdodge(),aes(shape=tile), stroke=1.5,
    colour="black",size=3) + geom_signif(aes(
      xmin= start,
      xmax= end,
      y_position = y_position,
      annotations = signif),
    data = time_sig , manual = TRUE, inherit.aes = FALSE, size=1, textsize = 10, tip_
length = 0) +
  facet_wrap(.~test, scales="free") + theme_bw() +
  ylab("Time near \n stimulus (s)") +
  theme(axis.title.x = element_blank(),
    axis.text.x = element_text(size=32, face= "bold", colour = "black"),
    axis.title.y = element_text(size=32, face= "bold"),
    axis.text.y = element_text(size=26),
    panel.grid.minor=element_blank(),
    panel.grid.major = element_blank(),
    axis.line = element_line(colour = "black", size=0.5),
    strip.background = element_blank(),
    panel.border = element_blank(),
    strip.text.x = element_blank()) +
  theme(legend.position = "none") +
  scale_shape_manual(values=c(17,15,19)) +
  scale_x_discrete(breaks=c("nb", "nbg","b"),
    labels=c("Non-breeding \n urine",
      "Non-breeding \n urine + glareosin", "Breeding \n urin
e")) +
  scale_y_continuous(expand = expansion(mult = c(0.05, .1))) +
  scale_fill_manual(values=c("#7DD6D4","#AE2AB1",'#E48100'))

time.gg

#add sniffing and time graph to same plot
glareosin_grid<-plot_grid(sniff.gg, time.gg, align="vh", ncol=1,
  hjust = -5,label_x = 0.03)

glareosin_grid

#save plot
png("/Users/user/Library/CloudStorage/OneDrive-Personal/PhD/Chapter 3 BV female respo
nse/Scientific Reports Paper/images/glareosin graph_reviewer comments.png", width = 4
2, height = 35, units= "cm",res=400)
glareosin_grid

dev.off()

```