Females are attracted to male urine

Holly Coombes

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```
#data
contact_bias<-read.csv("Females are attracted to male urine.csv", header=T)
```

```
#packages
library(tidyverse)
library(ggplot2)
library(lme4)
library(car)
library(emmeans)
library(cowplot)
library(gridExtra)
library(viridis)
library(ggsignif)
```

```
#check individuals visit tiles
contact_bias$subject<-as.factor(contact_bias$subject)
ggplot(data=contact_bias, aes(x=date, y=total.time.on.test.tile, group=subject, colou
r=subject)) +
  geom_point() + geom_line()

ggplot(data=contact_bias, aes(x=date, y=total.time.on.own.tile, group=subject, colour
=subject)) +
  geom_point() + geom_line()

#16686 does not spend time on either control or test tile in the majority of tests -
#only visits both tiles in a single test so removed from further analysis
contact_bias<-subset(contact_bias, subject != "16686")

#make stimulus factor
contact_bias$stimulus<-factor(contact_bias$stimulus, levels=c("Male", "Female", "Wate
r"))
```

```
#time sniffing stimulus LMM

#transforming raw data and recalculating bias scores
contact_bias$lg.sniffing.test.tile<-log(contact_bias$time.sniffing.test.tile +1)
contact_bias$lg.sniffing.own.tile<-log(contact_bias$time.sniffing.own.tile +1)
contact_bias$lg.sniff_bias<-contact_bias$lg.sniffing.test.tile -
  contact_bias$lg.sniffing.own.tile

#run linear mixed model on transformed data
contact.sniff.bias.lm<-lmer(lg.sniff_bias~stimulus+contact + (1|subject) + (1|date),
data=contact_bias)
summary(contact.sniff.bias.lm)
#check model plots

par(mfrow=c(2,2))
plot(resid(contact.sniff.bias.lm)~fitted(contact.sniff.bias.lm),ylab = 'Residuals', x
lab='Fitted values', main='Residual plot')
abline(h=0, lty=2)
hist(resid(contact.sniff.bias.lm),main='Histogram of residuals', xlab = 'Residuals')
qqnorm(resid(contact.sniff.bias.lm))
qqline(resid(contact.sniff.bias.lm))
mtext("Residual plots for bias in log time spent near stimulus comapred to own urin
e",cex.main=2,font=2, line=-1, outer=T)

#look for interaction effect
contact.sniff.bias2.lm<-lmer(lg.sniff_bias~stimulus*contact + (1|subject) + (1|date),
data=contact_bias)
Anova(contact.sniff.bias2.lm)
anova(contact.sniff.bias.lm, contact.sniff.bias2.lm)

#interaction non-significant and does not make model significantly better.

#model output
Anova(contact.sniff.bias.lm)
summary(contact.sniff.bias.lm)

#planned comparisons between male and female and male and water

#contrasts for planned comparisons between male and female urine
#and male urine and water
c1<- list(m_w=c(1,0,-1),
          m_f=c(.5,-.5,0))

contact_sniff_bias.em<-emmeans(contact.sniff.bias.lm, ~stimulus)
contrast(contact_sniff_bias.em, c1)

#post hoc contrast between female urine and water
pairs(contact_sniff_bias.em)
```

```
#Time near stimulus LMM

#transforming raw data and recalculating bias scores
contact_bias$lg.time.test.tile<-log(contact_bias$time.on.test.tile + 1)
contact_bias$lg.time.own.tile<-log(contact_bias$time.on.own.tile + 1)
contact_bias$lg.time_bias<-contact_bias$lg.time.test.tile -
  contact_bias$lg.time.own.tile

#LMM
contact.time.bias.lm<-lmer(lg.time_bias~stimulus+contact + (1|subject)+ (1|date), dat
a=contact_bias)
summary(contact.time.bias.lm)

#look at model plots
par(mfrow=c(2,2))
plot(resid(contact.time.bias.lm)~fitted(contact.time.bias.lm))
abline(h=0, lty=2)
hist(resid(contact.time.bias.lm))
qqnorm(resid(contact.time.bias.lm))
qqline(resid(contact.time.bias.lm))

#look at effect of interaction
contact.time.bias2.lm<-lmer(lg.time_bias~stimulus*contact + (1|subject)+ (1|date), da
ta=contact_bias)
summary(contact.time.bias2.lm)

anova(contact.time.bias.lm, contact.time.bias2.lm)
Anova(contact.time.bias2.lm)
#interaction not significant and doesn't improve model fit

#model outputs
summary(contact.time.bias.lm)
Anova(contact.time.bias.lm)

#post hoc contrasts male v female & male v water using same contrast matrix
#as sniffing model above.
contact_time_bias.em<-emmeans(contact.time.bias.lm, ~stimulus)
contrast(contact_time_bias.em, c1)
#significant difference between male and water and male and female urine

#post hoc contrast between female urine and water
pairs(contact_time_bias.em)
#no difference in time spent with female urine and water when investigation times exc
luded.
```

```
#effect sizes
se <- function(x) sqrt(var(x)/length(x))
averages<-contact_bias %>% group_by(stimulus) %>%
  summarise(mean_time=mean(time_bias), med_time=median(time_bias),
            se_time=se(time_bias), mean_sniff=mean(sniff_bias),
            med_sniff=median(sniff_bias), se_sniff=se(sniff_bias))
```

```
#fig 2 code

#time sniffing graph (as untransformed bias to test tile)
Fig_2a.gg<-ggplot(data=contact_bias, aes(x=stimulus, y=sniff_bias)) +
  geom_boxplot(aes(fill=contact), outlier.shape = NA) + theme_bw() +
  ylab("Time sniffing \n (test - own urine bias, s)") +
  geom_point(aes(shape=contact), position=position_jitterdodge(),
             stroke=1.5, colour="black", size=3) +
  theme(axis.title.x = element_blank(),
        axis.text.x = element_blank(),
        axis.ticks.x = element_blank(),
        axis.title.y = element_text(size=32, face= "bold",
        colour = "black",vjust=2,margin =margin(t=0, r=0, b=0, l=20)),
        axis.text.y = element_text(size=26),
        panel.border = element_blank(), panel.grid.minor=element_blank(),
        panel.grid.major = element_blank(),
        axis.line = element_line(colour = "black", size=0.5),
        axis.line.x = element_blank(), legend.text = element_text(size=24,
        face= "bold", colour = "black"),
        legend.key.size = unit(2,'cm')) +
  geom_hline(yintercept=0, linetype="dashed", color="black", size=1)+
  scale_fill_manual(values=c("#43a2ca","#a8ddb5"),
    name="", breaks=c("n", "y"),
                    labels=c("No contact", "Contact")) +
  scale_shape_manual(values=c(15,17), breaks=c("n", "y"),
                     labels=c("No contact", "Contact"), name="") +
  scale_x_discrete(name="Test stimulus", breaks=c("Male", "Female", "Water"),
                   labels=c("Male urine", "Female urine", "Water")) +
  scale_y_continuous(expand = expansion(mult = c(0.05, .1)))

#add significance
sniffing_sig <- data.frame(signif = c("***", "*", "*"),
                           y_position = c(95,80, 70),
                           group = c(1,2,3),
                          start = c("Male", "Male", 'Female'),
                         end = c("Water", "Female", 'Water'))

Fig_2a.gg<-Fig_2a.gg + geom_signif(aes(
                  xmin= start,
                  xmax= end,
                  y_position = y_position,
                  annotations = signif),
    data = sniffing_sig , manual = TRUE, inherit.aes = FALSE, size=1, textsize = 10,
tip_length = 0)

#time nearby (as untransformed bias to test tile)
Fig_2b.gg<-ggplot(data=contact_bias, aes(x=stimulus, y=time_bias)) +
  geom_boxplot(aes(fill=contact), outlier.shape = NA) + theme_bw() +
  ylab("Time nearby not sniffing \n (test - own urine bias, s)") +
  geom_point(aes(shape=contact),position=position_jitterdodge(), size=3,
             stroke=1.5,colour="black") +
  theme(axis.title.x = element_text(size=32, face="bold", colour="black",
                                    margin =margin(t=0, r=0, b=10, l=0), vjust=-1),
        axis.text.x = element_text(size=28, colour="black"),
        axis.ticks.x = element_blank(),
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        axis.title.y = element_text(size=32, face= "bold", colour = "black",
                                    vjust=2,
                                    margin =margin(t=0, r=0, b=0, l=10)),
        axis.text.y = element_text(size=26),
        panel.border = element_blank(), panel.grid.minor=element_blank(),
        panel.grid.major = element_blank(),
        axis.line = element_line(colour = "black", size=0.5),
        axis.line.x = element_blank(),
        legend.position = "none") +
  geom_hline(yintercept=0, linetype="dashed", color="black", size=1) +
  scale_fill_manual(values=c("#43a2ca","#a8ddb5"), name="", breaks=c("n", "y"),
                    labels=c("No contact", "Contact")) +
  scale_shape_manual(values=c(15,17), breaks=c("n", "y"),
                     labels=c("No contact", "Contact"), name="") +
  scale_x_discrete(name="Test stimulus", breaks=c("Male", "Female", "Water"),
                   labels=c("Male urine", "Female urine", "Water")) +
  scale_y_continuous(expand = expansion(mult = c(.05, .06)))

#add significance
time_sig <- data.frame(signif = c("*", "*", "NS"),
                           y_position = c(200,170,150),
                           group = c(1,2,3),
                          start = c("Male", "Male", 'Female'),
                         end = c("Water", "Female", 'Water'))

Fig_2b.gg<-Fig_2b.gg + 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)

#get legend
Fig2_legend<-get_legend(Fig_2a.gg)
#remove legend from sniffing graph
Fig_2a.gg2<-Fig_2a.gg + theme(legend.position = "none")


#plot on grid
Fig_2<-plot_grid(Fig_2a.gg2, Fig_2b.gg, align="vh",
                         labels = c('a', 'b'),label_size = 28,
                         ncol=1, label_x = .175, label_y = 1.02)

#save plot
png("Figure 2.png", width = 40, height = 40, units = 'cm', res = 300)
grid.arrange(arrangeGrob(Fig_2, Fig2_legend, ncol=2, widths= c(1,0.3))) # Make plot
dev.off()
```