

qpCR-Figures

C-T Berezin

10/30/2021

```
library(tidyverse)

## -- Attaching packages ----- tidyverse 1.3.1 --

## v ggplot2 3.3.5      v purrr 0.3.4
## v tibble 3.1.5       v dplyr 1.0.7
## v tidyr 1.1.4        v stringr 1.4.0
## v readr 2.0.2        v forcats 0.5.1

## -- Conflicts ----- tidyverse_conflicts() --
## x dplyr::filter() masks stats::filter()
## x dplyr::lag()      masks stats::lag()

library(ggplot2)
library(ggthemes)
library(forcats)
library(ggpubr)
library(writexl)
```

Is POMC under circadian regulation?

```
pomc_circ <- read.csv("../data/08202021-POMC-final.csv", fileEncoding = 'UTF-8-BOM')
pomc_circ <- pomc_circ %>% mutate(Time = as.factor(Time),
                                   Sex = as.factor(Sex)) %>%
  mutate(Time = fct_relevel(Time, c("8am", "4pm", "12am"))) %>%
  mutate(Time = fct_recode(Time,
                            "08:00" = "8am",
                            "16:00" = "4pm",
                            "00:00" = "12am")) %>%
  filter(Relative.GE < 3) #remove mouse with high relative GE (data not normal)

#p-values from SigmaPlot
pomc_circ_stats <- tibble::tribble(
  ~group1, ~group2, ~group3, ~p.adj,
  "08:00", "16:00", "00:00", "0.865"
)
```

```

pomc_circ_plot <- ggplot(pomc_circ, aes(x=Time, y=Relative.GE)) +
  geom_point(aes(col=Sex)) +
  stat_summary(fun = 'mean', geom="bar", aes(alpha=0.5)) +
  stat_summary(fun.data = mean_se, geom = "errorbar", width=.1, alpha=0.5) +
  theme_few() +
  theme(legend.position = "none") +
  labs(x="Time (Hour)",
       y="Relative Gene Expression",
       title = "Figure 3",
       subtitle = "Expression of POMC mRNA") +
  geom_bracket(xmin="08:00", xmax="00:00",
              label=pomc_circ_stats$p.adj,
              data=pomc_circ_stats,
              y.position=2.5) +
  scale_y_continuous(expand = expansion(mult = c(0, 0.1)))

ggsave(filename="../figures/pomc_circ_sex.png", plot=pomc_circ_plot, height=3, width=3)

```

Is POMC expression light-driven?

```

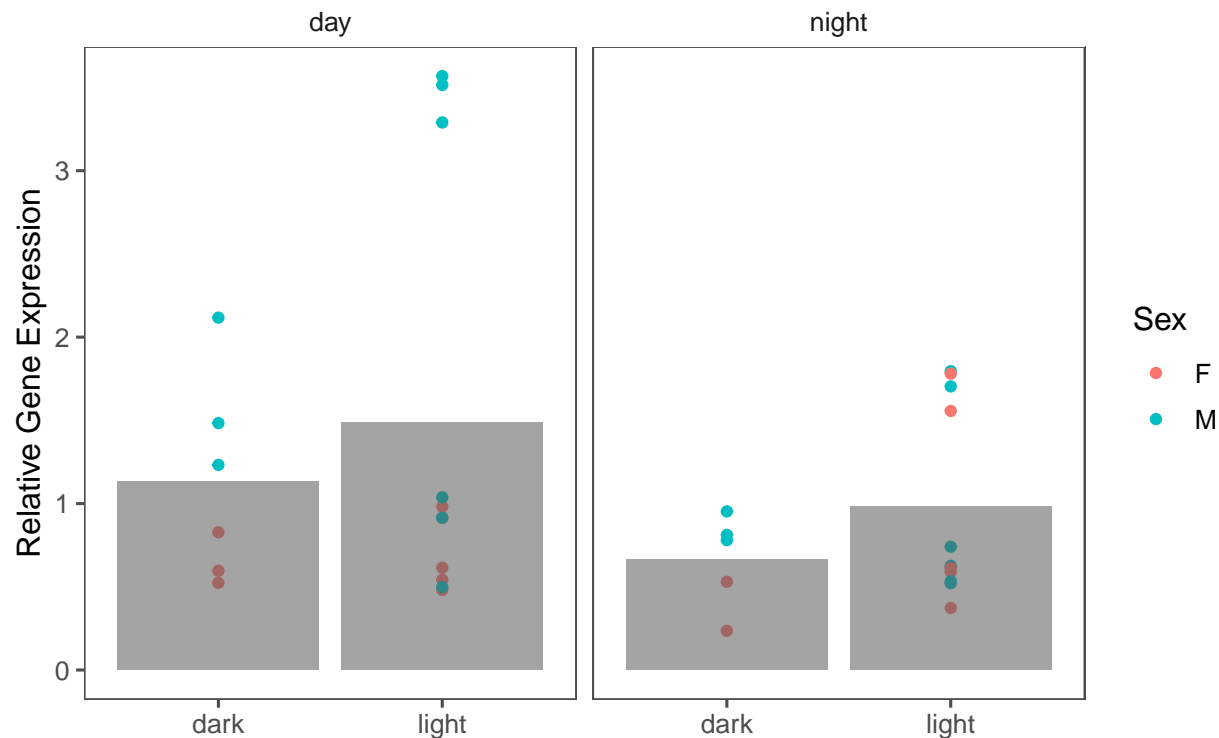
pomc_ld <- read.csv("../data/01142022-POMC-light-dark-add'l-samps.csv", fileEncoding = 'UTF-8-BOM')
pomc_ld <- pomc_ld %>% mutate(Time = as.factor(Time.of.Day),
                             Light = as.factor(Light.Condition),
                             Sex = as.factor(Sex)) %>%
  dplyr::select(-Time.of.Day, -Light.Condition)

pomc_ld_plot <- ggplot(pomc_ld, aes(x=Light, y=Relative.G.E.)) +
  geom_point(aes(col=Sex)) +
  theme_few() +
  facet_wrap(~Time) +
  stat_summary(fun = 'mean', geom="bar", aes(alpha=0.5)) +
  labs(x="",
       y="Relative Gene Expression",
       title = "POMC mRNA Expression") +
  scale_alpha(guide = "none")

pomc_ld_plot

```

POMC mRNA Expression



```
ggsave(filename="../figures/pomc_ld_add'l.png", plot=pomc_ld_plot, height=3, width=3)
```

```
t.test(Relative.G.E. ~ Sex, pomc_ld)
```

```
##
## Welch Two Sample t-test
##
## data: Relative.G.E. by Sex
## t = -2.6484, df = 23.38, p-value = 0.01425
## alternative hypothesis: true difference in means between group F and group M is not equal to 0
## 95 percent confidence interval:
## -1.2590046 -0.1553002
## sample estimates:
## mean in group F mean in group M
## 0.7441239 1.4512763
```

```
pomc_ld_lm <- lm(Relative.G.E. ~ Sex*Light*Time, pomc_ld)
anova(pomc_ld_lm)
```

```
## Analysis of Variance Table
##
## Response: Relative.G.E.
##
##           Df Sum Sq Mean Sq F value Pr(>F)
## Sex       1  4.0914  4.0914  6.9085 0.01446 *
```

```
## Light      1  0.7450  0.7450  1.2580  0.27270
## Time       1  2.1772  2.1772  3.6763  0.06669 .
## Sex:Light   1  0.0008  0.0008  0.0014  0.97091
## Sex:Time    1  2.5523  2.5523  4.3096  0.04834 *
## Light:Time   1  0.0015  0.0015  0.0025  0.96038
## Sex:Light:Time 1  0.3867  0.3867  0.6530  0.42667
## Residuals   25 14.8058  0.5922
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
pomc_ld_av <- aov(pomc_ld_lm)
TukeyHSD(pomc_ld_av)
```

```
## Tukey multiple comparisons of means
## 95% family-wise confidence level
##
## Fit: aov(formula = pomc_ld_lm)
##
## $Sex
##          diff          lwr          upr          p adj
## M-F 0.7071524 0.153048 1.261257 0.0144557
##
## $Light
##          diff          lwr          upr          p adj
## light-dark 0.3187344 -0.2665485 0.9040173 0.2726958
##
## $Time
##          diff          lwr          upr          p adj
## night-day -0.5131982 -1.065262 0.03886529 0.0670614
##
## $'Sex:Light'
##          diff          lwr          upr          p adj
## M:dark-F:dark 0.7211503 -0.56063890 2.0029395 0.4255743
## F:light-F:dark 0.3301872 -0.82923474 1.4896092 0.8612768
## M:light-F:dark 1.0303407 -0.09641495 2.1570963 0.0818368
## F:light-M:dark -0.3909631 -1.48407659 0.7021504 0.7598424
## M:light-M:dark 0.3091904 -0.74921224 1.3675930 0.8521175
## M:light-F:light 0.7001534 -0.20620839 1.6065153 0.1728438
##
## $'Sex:Time'
##          diff          lwr          upr          p adj
## M:day-F:day 1.26278520 0.2342028 2.29136764 0.0120273
## F:night-F:day 0.09714678 -0.9984040 1.19269755 0.9947655
## M:night-F:day 0.24218992 -0.7863925 1.27077236 0.9153692
## F:night-M:day -1.16563842 -2.2324080 -0.09886887 0.0284881
## M:night-M:day -1.02059528 -2.0184668 -0.02272374 0.0436577
## M:night-F:night 0.14504315 -0.9217264 1.21181269 0.9817488
##
## $'Light:Time'
##          diff          lwr          upr          p adj
## light:day-dark:day 0.3295261 -0.7447932 1.4038454 0.8331289
## dark:night-dark:day -0.5320491 -1.8138383 0.7497401 0.6678291
## light:night-dark:day -0.1757383 -1.2500577 0.8985810 0.9690230
## dark:night-light:day -0.8615752 -2.0032950 0.2801446 0.1885459
```

```
## light:night-light:day -0.5052644 -1.4078732 0.3973443 0.4299273
## light:night-dark:night 0.3563108 -0.7854090 1.4980306 0.8259208
##
## $'Sex:Light:Time'
##
##               diff          lwr          upr          p adj
## M:dark:day-F:dark:day 0.961792839 -1.1118752 3.0354609 0.7842025
## F:light:day-F:dark:day 0.057760479 -1.7969846 1.9125056 1.0000000
## M:light:day-F:dark:day 1.488094717 -0.3077545 3.2839439 0.1583872
## F:dark:night-F:dark:day -0.266075163 -2.5845065 2.0523562 0.9999303
## M:dark:night-F:dark:day 0.199019353 -1.8746487 2.2726874 0.9999792
## F:light:night-F:dark:day 0.333603409 -1.5211417 2.1883485 0.9986579
## M:light:night-F:dark:day 0.337890204 -1.4579590 2.1337394 0.9982137
## F:light:day-M:dark:day -0.904032359 -2.7587774 0.9507127 0.7410782
## M:light:day-M:dark:day 0.526301878 -1.2695473 2.3221511 0.9751004
## F:dark:night-M:dark:day -1.227868002 -3.5462994 1.0905633 0.6580686
## M:dark:night-M:dark:day -0.762773486 -2.8364415 1.3108946 0.9201432
## F:light:night-M:dark:day -0.628189430 -2.4829345 1.2265556 0.9466955
## M:light:night-M:dark:day -0.623902635 -2.4197518 1.1719466 0.9394779
## M:light:day-F:light:day 1.430334237 -0.1075391 2.9682076 0.0817383
## F:dark:night-F:light:day -0.323835643 -2.4487131 1.8010418 0.9995401
## M:dark:night-F:light:day 0.141258874 -1.7134862 1.9960040 0.9999957
## F:light:night-F:light:day 0.275842930 -1.3304134 1.8820993 0.9990020
## M:light:night-F:light:day 0.280129724 -1.2577437 1.8180031 0.9985453
## F:dark:night-M:light:day -1.754169880 -3.8278379 0.3194982 0.1421939
## M:dark:night-M:light:day -1.289075364 -3.0849246 0.5067738 0.2984509
## F:light:night-M:light:day -1.154491308 -2.6923647 0.3833821 0.2500589
## M:light:night-M:light:day -1.150204513 -2.6165092 0.3161002 0.2064938
## M:dark:night-F:dark:night 0.465094516 -1.8533368 2.7835259 0.9973289
## F:light:night-F:dark:night 0.599678572 -1.5251989 2.7245560 0.9797791
## M:light:night-F:dark:night 0.603965367 -1.4697027 2.6776334 0.9759316
## F:light:night-M:dark:night 0.134584056 -1.7201610 1.9893291 0.9999970
## M:light:night-M:dark:night 0.138870851 -1.6569783 1.9347201 0.9999953
## M:light:night-F:light:night 0.004286795 -1.5335866 1.5421602 1.0000000
```

```
#males only
```

```
pomc_ld_m <- pomc_ld %>% filter(Sex == "M")
```

```
pomc_ld_m_lm <- lm(Relative.G.E. ~ Light*Time, pomc_ld_m)
```

```
anova(pomc_ld_m_lm)
```

```
## Analysis of Variance Table
```

```
##
```

```
## Response: Relative.G.E.
```

```
##           Df Sum Sq Mean Sq F value Pr(>F)
```

```
## Light      1  0.4425  0.4425  0.4810 0.49931
```

```
## Time       1  4.6915  4.6915  5.1005 0.04041 *
```

```
## Light:Time 1  0.1501  0.1501  0.1632 0.69234
```

```
## Residuals 14 12.8776  0.9198
```

```
## ---
```

```
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
pomc_ld_m_av <- aov(pomc_ld_m_lm)
```

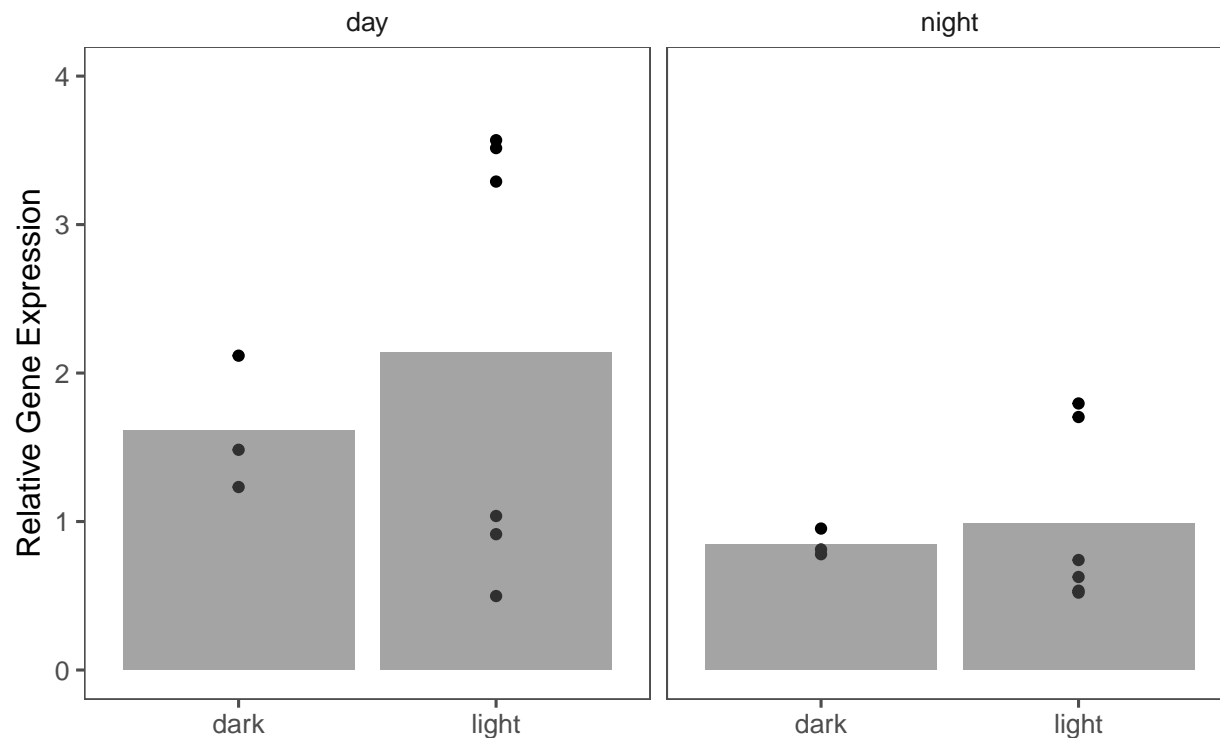
```
TukeyHSD(pomc_ld_m_av)
```

```
## Tukey multiple comparisons of means
## 95% family-wise confidence level
##
## Fit: aov(formula = pomc_ld_m_lm)
##
## $Light
##           diff           lwr           upr           p adj
## light-dark 0.3325864 -0.6959205 1.361093 0.499307
##
## $Time
##           diff           lwr           upr           p adj
## night-day -1.021061 -1.990746 -0.05137526 0.0404069
##
## $'Light:Time'
##           diff           lwr           upr           p adj
## light:day-dark:day 0.5263019 -1.444842 2.4974462 0.8639582
## dark:night-dark:day -0.7627735 -3.038855 1.5133079 0.7660601
## light:night-dark:day -0.6239026 -2.595047 1.3472417 0.7947565
## dark:night-light:day -1.2890754 -3.260220 0.6820690 0.2715606
## light:night-light:day -1.1502045 -2.759637 0.4592281 0.2079303
## light:night-dark:night 0.1388709 -1.832273 2.1100152 0.9968153
```

```
pomc_ld_m_plot <- ggplot(pomc_ld_m, aes(x=Light, y=Relative.G.E.)) +
  geom_point() +
  theme_few() +
  facet_wrap(~Time) +
  stat_summary(fun = 'mean', geom="bar", aes(alpha=0.5)) +
  labs(x="",
       y="Relative Gene Expression",
       title = "POMC mRNA in Males") +
  scale_alpha(guide = "none") +
  scale_y_continuous(limits = c(0,4))

pomc_ld_m_plot
```

POMC mRNA in Males



```
ggsave(filename="../figures/pomc_ld_add'l_m_only.png", plot=pomc_ld_m_plot, height=3, width=3)
```

```
#females only
```

```
pomc_ld_f <- pomc_ld %>% filter(Sex == "F")
```

```
pomc_ld_f_lm <- lm(Relative.G.E. ~ Light*Time, pomc_ld_f)
```

```
anova(pomc_ld_f_lm)
```

```
## Analysis of Variance Table
```

```
##
```

```
## Response: Relative.G.E.
```

```
##          Df Sum Sq Mean Sq F value Pr(>F)
```

```
## Light      1  0.30424  0.304239   1.7356 0.2145
```

```
## Time       1  0.03706  0.037063   0.2114 0.6546
```

```
## Light:Time  1  0.23812  0.238115   1.3584 0.2685
```

```
## Residuals 11  1.92827  0.175297
```

```
pomc_ld_f_av <- aov(pomc_ld_f_lm)
```

```
TukeyHSD(pomc_ld_f_av)
```

```
## Tukey multiple comparisons of means
```

```
## 95% family-wise confidence level
```

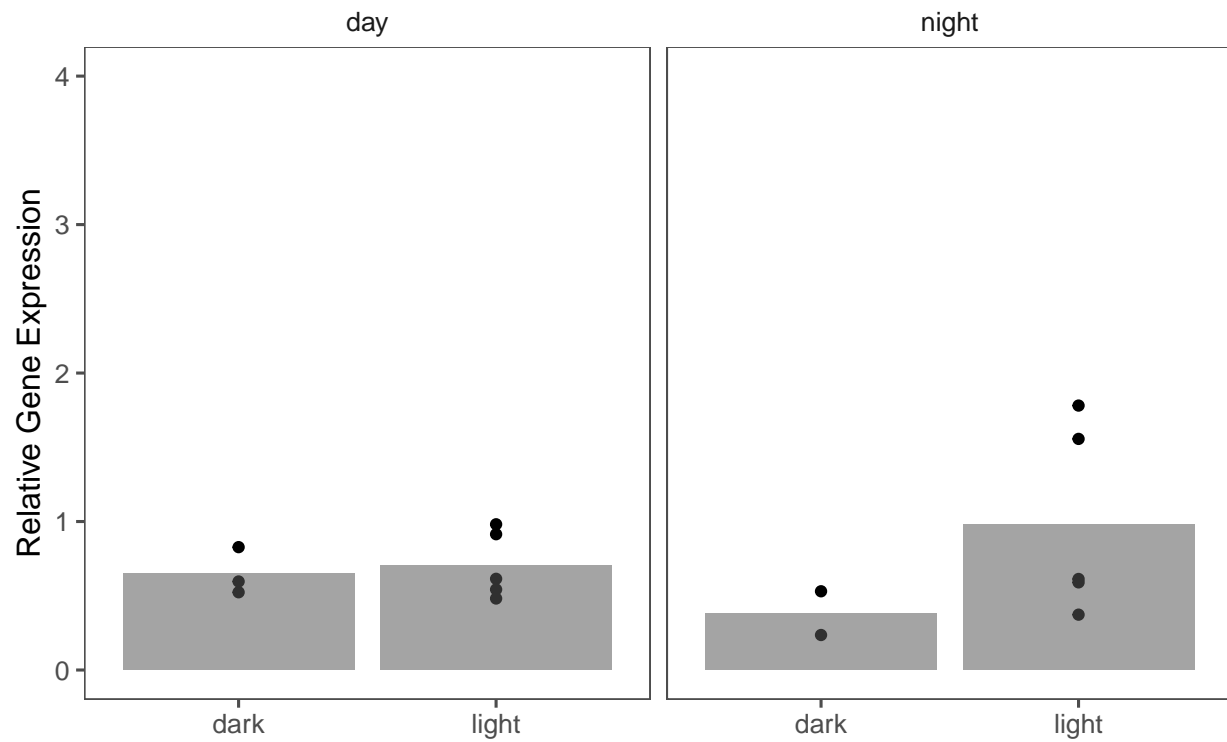
```
##
```

```
## Fit: aov(formula = pomc_ld_f_lm)
##
## $Light
##           diff           lwr           upr           p adj
## light-dark 0.302112 -0.2026247 0.8068487 0.2144828
##
## $Time
##           diff           lwr           upr           p adj
## night-day 0.09919209 -0.3777393 0.5761235 0.6560342
##
## $'Light:Time'
##           diff           lwr           upr           p adj
## light:day-dark:day 0.05776048 -0.8624513 0.9779722 0.9974614
## dark:night-dark:day -0.26607516 -1.4163399 0.8841895 0.8964363
## light:night-dark:day 0.33360341 -0.5866083 1.2538152 0.7019107
## dark:night-light:day -0.32383564 -1.3780706 0.7303994 0.7926629
## light:night-light:day 0.27584293 -0.5210838 1.0727697 0.7296399
## light:night-dark:night 0.59967857 -0.4545564 1.6539136 0.3630521
```

```
pomc_ld_f_plot <- ggplot(pomc_ld_f, aes(x=Light, y=Relative.G.E.)) +
  geom_point() +
  theme_few() +
  facet_wrap(~Time) +
  stat_summary(fun = 'mean', geom="bar", aes(alpha=0.5)) +
  labs(x="",
       y="Relative Gene Expression",
       title = "POMC mRNA in Females") +
  scale_alpha(guide = "none") +
  scale_y_continuous(limits = c(0,4))
```

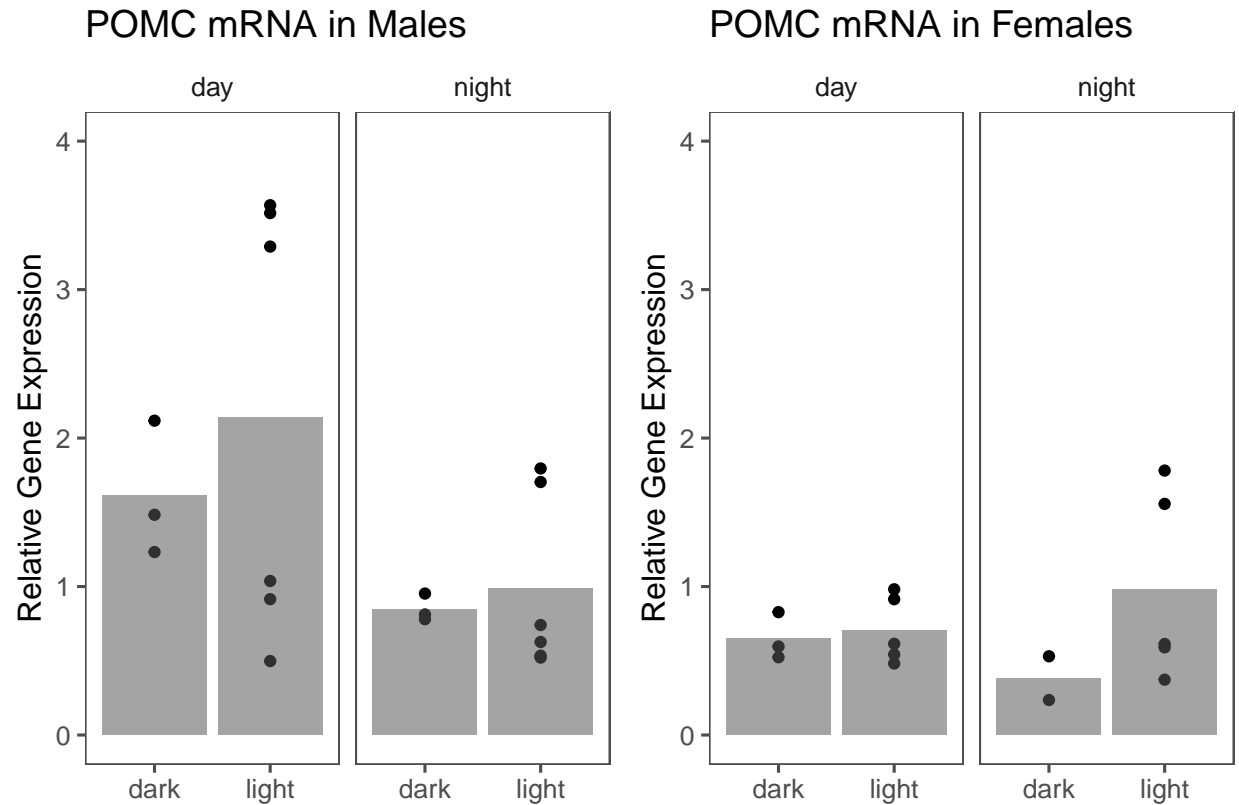
```
pomc_ld_f_plot
```


POMC mRNA in Females



```
ggsave(filename="../figures/pomc_ld_add'l_f_only.png", plot=pomc_ld_f_plot, height=3, width=3)
```

```
bothpointplots <- ggarrange(pomc_ld_m_plot, pomc_ld_f_plot, nrow=1)  
bothpointplots
```



McKO validation

```
mcko <- read.csv("../data/08162021-McKO-totalret-final.csv", fileEncoding = 'UTF-8-BOM')
mcko <- mcko %>%
  mutate(Genotype = fct_relevel(Genotype, c("WT", "McKO")))

#p-values from SigmaPlot
mcko_stats <- tibble::tribble(
  ~group1, ~group2, ~p.adj,
  "WT", "McKO", "<0.001"
)

mcko_plot <- mcko %>%
  ggplot(aes(x=Genotype, y=Relative.GE)) +
  geom_point() +
  stat_summary(fun = 'mean', geom="bar", aes(alpha=0.5), fill="darkorange") +
  stat_summary(fun.data = mean_se, geom = "errorbar", width=.1, alpha=0.5) +
  theme_few() +
  theme(legend.position = "none") +
  labs(y="Relative Gene Expression",
       title="Expression of MOR mRNA") +
  stat_pvalue_manual(mcko_stats,
    y.position = 1.5, step.increase = 0.1,
    label = "p.adj") +
```

```

  scale_y_continuous(expand = expansion(mult = c(0, 0.1)))
ggsave(filename="../figures/mcko_fig_bars_orange.png", plot=mcko_plot, height=4, width=3)

```

MOR mRNA in morphine vs saline treatment (retinas only)

```

treatment <- c("saline", "saline", "saline", "saline",
               "morphine", "morphine", "morphine", "morphine")
sample_num <- c("1", "2", "3", "4",
               "1", "2", "3", "4")
rge <- c(1.025,
        1.054,
        1.12,
        0.822,
        0.677,
        1.054,
        0.938,
        1.057)
mor <- tibble(treatment, sample_num, rge)
mor <- mor %>%
  mutate(treatment = fct_relevel(treatment, c("saline", "morphine")))

#p-value from SigmaPlot
mor_stats <- tibble::tribble(
  ~group1, ~group2, ~p.adj,
  "saline", "morphine", "0.528"
)

mor_plot <- mor %>% ggplot(aes(x=treatment, y=rge)) +
  stat_summary(fun = 'mean', geom="bar", fill="grey40") +
  stat_summary(fun.data = mean_se, geom = "errorbar", width=.1, alpha=0.5) +
  geom_point() +
  labs(title = "Expression of MOR mRNA",
       x="",
       y="Relative Gene Expression") +
  theme_few() +
  scale_y_continuous(expand = expansion(mult = c(0, 0.1))) +
  stat_pvalue_manual(mor_stats,
    y.position = 1.25, step.increase = 0.1,
    label = "p.adj")

ggsave(filename="../figures/mor_morphine_qpcr.png", plot=mor_plot, height=4, width=3)

```