

# qpCR-Figures

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```
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?

```

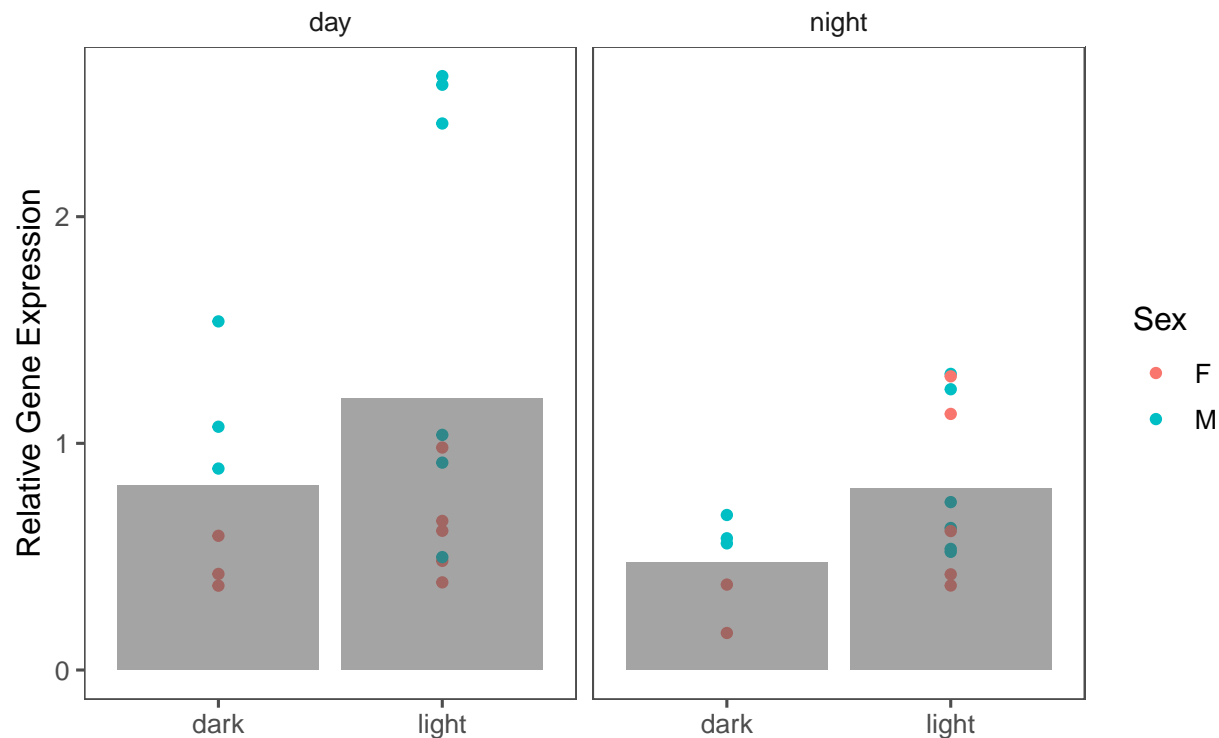
pomc_ld <- read.csv("../data/01142022-POMC-light-dark-add'l-samps.csv", fileEncoding = 'UTF-8-BOM')
pomc_ld <- pomc_ld %>% mutate(Time = as.factor(Time.of.Day),
                             Light = as.factor(Light.Condition),
                             Sex = as.factor(Sex)) %>%
  select(-Time.of.Day, -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 = -2.8855, df = 24.229, p-value = 0.008085
## alternative hypothesis: true difference in means between group F and group M is not equal to 0
## 95 percent confidence interval:
## -0.9236976 -0.1535643
## sample estimates:
## mean in group F mean in group M
## 0.5922312 1.1308621
```

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

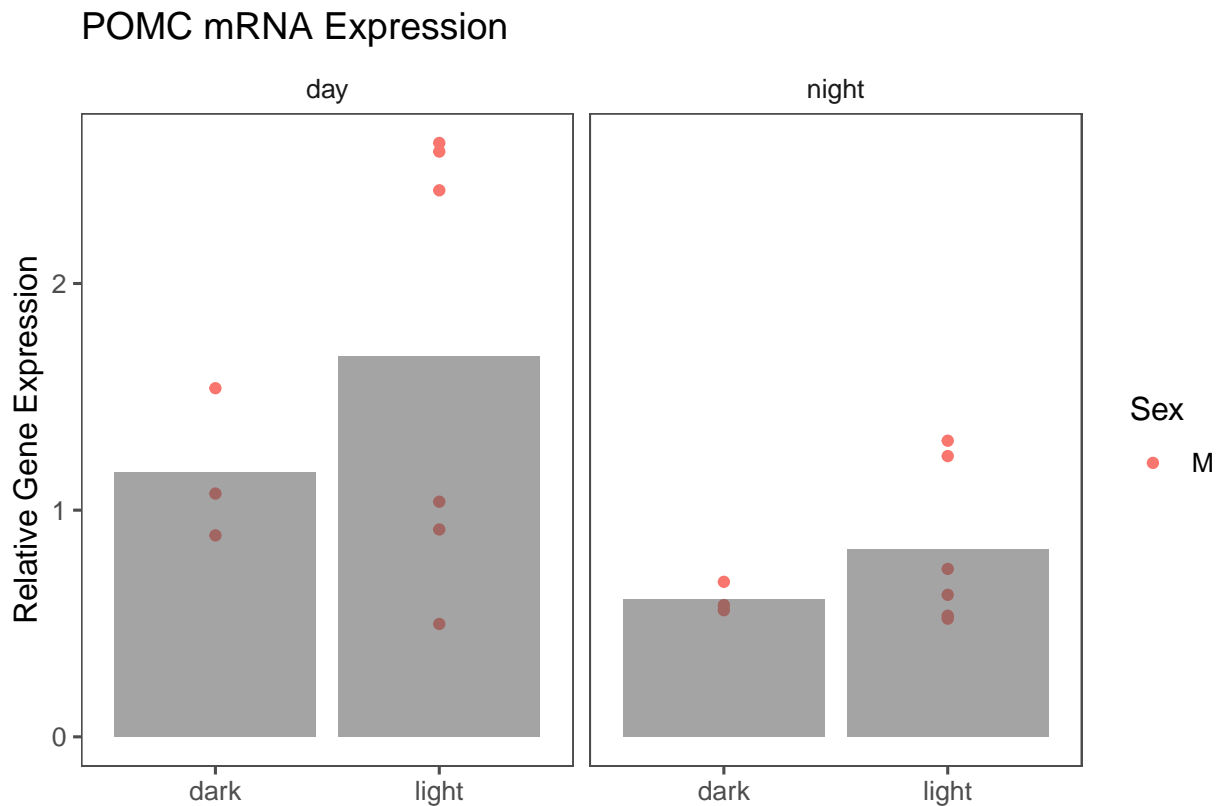
```
## Analysis of Variance Table
##
## Response: Relative.G.E.
##              Df Sum Sq Mean Sq F value    Pr(>F)
## Sex           1  2.3737  2.37374   9.1999 0.005575 **
```

```
## Light      1 0.8484 0.84838 3.2881 0.081809 .
## Time      1 1.3021 1.30215 5.0467 0.033749 *
## Sex:Light  1 0.0004 0.00045 0.0017 0.967061
## Sex:Time   1 1.2549 1.25489 4.8636 0.036848 *
## Light:Time 1 0.0002 0.00020 0.0008 0.978159
## Sex:Light:Time 1 0.1752 0.17516 0.6789 0.417773
## Residuals 25 6.4504 0.25802
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

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

pomc_ld_m_plot <- ggplot(pomc_ld_m, 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_m_plot
```



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

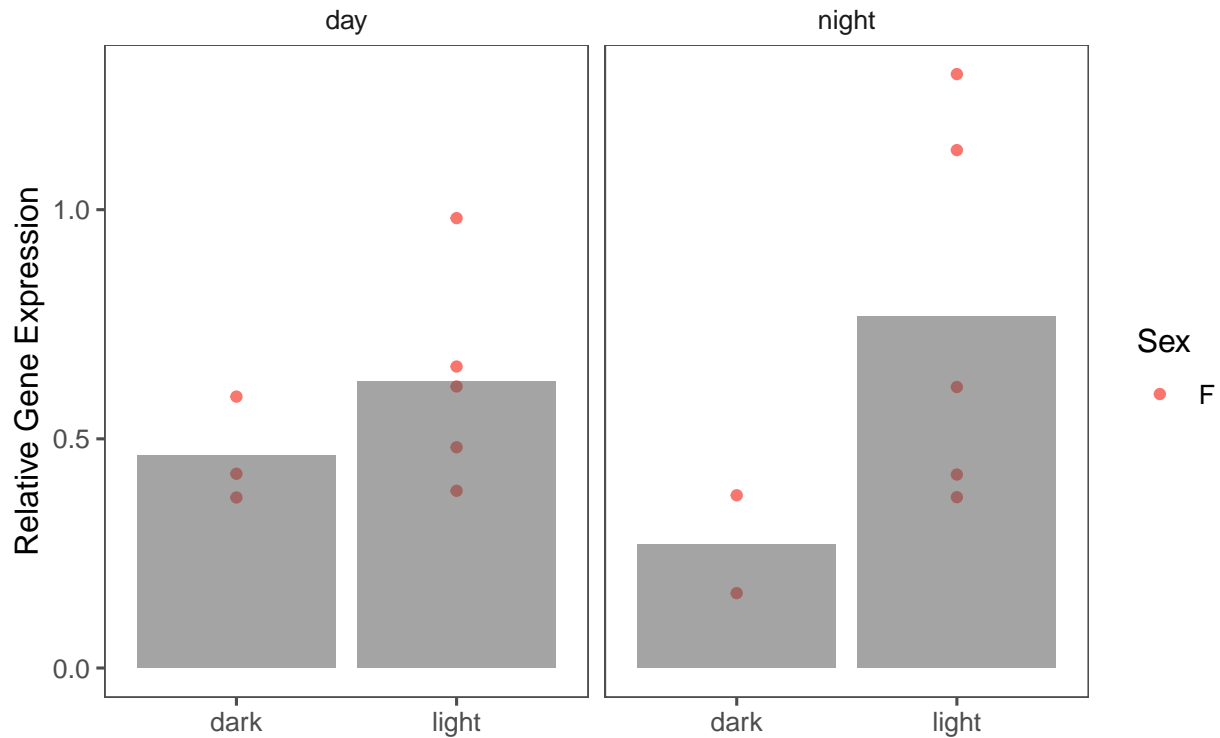
```
## Analysis of Variance Table
##
## Response: Relative.G.E.
##           Df Sum Sq Mean Sq F value    Pr(>F)
## Light      1  0.5339  0.53394    1.3625  0.26259
## Time       1  2.5477  2.54768    6.5013  0.02313 *
## Light:Time  1  0.0844  0.08438    0.2153  0.64975
## Residuals 14  5.4862  0.39187
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

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

pomc_ld_f_plot <- ggplot(pomc_ld_f, 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_f_plot
```

## POMC mRNA Expression



```
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  0.32004   0.32004    3.6509 0.08244 .
## Time       1  0.00421   0.00421    0.0480 0.83063
## Light:Time  1  0.09097   0.09097    1.0378 0.33022
## Residuals 11  0.96425   0.08766
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

## 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", "McK0", "<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)
```