

# 10302021-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 3.1.6       v dplyr 1.0.7
## v tidyr 1.1.4        v stringr 1.4.0
## v readr 2.1.0        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)
```

## Is POMC under circadian regulation?

```
pomc <- read.csv("../data/08202021-POMC-final.csv", fileEncoding = 'UTF-8-BOM')
pomc <- pomc %>% mutate(Time = as.factor(Time)) %>%
  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_stats <- tibble::tribble(
  ~group1, ~group2, ~group3, ~p.adj,
  "08:00", "16:00", "00:00", "0.865"
)

pomc_plot <- ggplot(pomc, aes(x=Time, 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(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_stats$p.adj, data=pomc_stats, y.position=2.5) +
  scale_y_continuous(expand = expansion(mult = c(0, 0.1)))

ggsave(filename="../figures/pomc_fig_w_bars.png", plot=pomc_plot, height=3, width=3)

```

## 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)

```

### p-glycoprotein (Abcb1a) expression

```

pgp <- read.csv("../data/Abcb1a-qpcr-results.csv", fileEncoding = 'UTF-8-BOM')
pgp

```

##	Relative.GE	Sample	Tissue	Treatment
## 1	2.1416193	M1-hyp	hypothalamus	morphine
## 2	2.3189154	M2-hyp	hypothalamus	morphine
## 3	2.1488628	M3-hyp	hypothalamus	morphine
## 4	1.7935729	M4-hyp	hypothalamus	morphine
## 5	1.0317396	M5-hyp	hypothalamus	morphine
## 6	1.3912871	M6-hyp	hypothalamus	morphine
## 7	1.2963135	M7-hyp	hypothalamus	morphine
## 8	1.8567608	S1-hyp	hypothalamus	saline
## 9	1.9882538	S2-hyp	hypothalamus	saline
## 10	1.9258292	S3-hyp	hypothalamus	saline
## 11	1.6402439	S4-hyp	hypothalamus	saline
## 12	1.6582910	S5-hyp	hypothalamus	saline
## 13	1.3635515	S6-hyp	hypothalamus	saline
## 14	3.3034537	S7-hyp	hypothalamus	saline
## 15	2.4951849	S8-hyp	hypothalamus	saline
## 16	3.0261063	S9-hyp	hypothalamus	saline
## 17	0.7962585	M1-ret	retina	morphine
## 18	1.1283773	M2-ret	retina	morphine
## 19	0.9744662	M3-ret	retina	morphine
## 20	1.1761635	M4-ret	retina	morphine
## 21	1.0656946	M5-ret	retina	morphine
## 22	0.8076524	M6-ret	retina	morphine
## 23	0.8788282	M7-ret	retina	morphine
## 24	0.8522861	S1-ret	retina	saline
## 25	1.0590154	S2-ret	retina	saline

```

## 26  1.0892752 S3-ret      retina    saline
## 27  1.2027263 S4-ret      retina    saline
## 28  0.8925252 S5-ret      retina    saline
## 29  1.1861894 S6-ret      retina    saline
## 30  0.8358540 S7-ret      retina    saline
## 31  0.8157816 S8-ret      retina    saline
## 32  1.1763925 S9-ret      retina    saline

pgp_plot <- pgp %>% ggplot(aes(x=Treatment, y=Relative.GE)) +
  geom_point() +
  facet_wrap(~fct_relevel(Tissue, c("retina", "hypothalamus"))) +
  labs(y="Relative Gene Expression")

ggsave(filename = "../figures/pgp_plot.png", plot = pgp_plot, height=4, width=6)

```