Analysis of B-endorphin/ChAT IHC Count Data

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```
library(knitr)
library(tidyverse)
library(dplyr)
library(WebPower)
library(ggpubr)
library(car)
library(ggthemes)
library(viridis)
```

Reading in and Tidying the Data

```
counts <- read_csv("../data/B-endo-totals.csv")
```

```
## Rows: 12 Columns: 12
```

```
## -- Column specification --------------------------------------------------------
## Delimiter: ","
## chr (3): Light, Time, Sex
## dbl (9): Mouse, ChAT, Bendo, Percentage, Age, GCLchat, GCLbendo, INLchat, IN...
```

```
##
## i Use `spec()` to retrieve the full column specification for this data.
## i Specify the column types or set `show_col_types = FALSE` to quiet this message.
```

```
counts <- counts %>%
  dplyr::select(-ChAT, -Bendo, -Percentage) %>%
  #make columns lowercase
  rename(mouse = Mouse,
         light = Light,
         time = Time,
         sex = Sex,
         age = Age,
         GCL_chat = GCLchat,
         GCL_bendo = GCLbendo,
         INL_chat = INLchat,
         INL_bendo = INLbendo) %>%
  #update the classes of some columns
```

```
  mutate(mouse = as.character(mouse),
         light = factor(as.factor(light), levels=c("light", "dark")),
         time = as.factor(time),
  #reintroduce total counts
         total_chat = GCL_chat+INL_chat,
         total_bendo = GCL_bendo+INL_bendo) %>%
  #calculate percentages
  mutate(GCL_perc=GCL_bendo/GCL_chat*100,
         INL_perc=INL_bendo/INL_chat*100,
         total_perc=total_bendo/total_chat*100)
counts
```

```
## # A tibble: 12 x 14
##    mouse light time  sex     age GCL_chat GCL_bendo INL_chat INL_bendo
##    <chr> <fct> <fct> <chr> <dbl>    <dbl>     <dbl>    <dbl>     <dbl>
##  1 11    light day   F        95     4427       139     5589       416
##  2 5     light day   M       141     1646       124     2013       157
##  3 32    light day   M        65     2722       150     3232       194
##  4 57    dark  night F        54     2474       173     3083       394
##  5 47    dark  night F        97     4374       385     5134      1104
##  6 20    dark  night F       122     5088       272     5886      1075
##  7 31    dark  day   M        70     4515       537     4730      1575
##  8 34    dark  day   F        70     4113       505     4587      1005
##  9 97    dark  day   M        74     4373       462     5313       836
## 10 92    light night F        96     4221       501     5266      1347
## 11 44    light night M        41     4986       465     5242      1087
## 12 93    light night F        96     3138       225     3672       348
## # ... with 5 more variables: total_chat <dbl>, total_bendo <dbl>,
## #   GCL_perc <dbl>, INL_perc <dbl>, total_perc <dbl>
```

```
#data setup for previous anova analysis
#also used in manova shapiro tests for ease
anovpercs <- counts %>%
  #give one row for each GCL, INL, and total
  #retain only the percentages, not the raw counts
  dplyr::select(-c(GCL_chat:total_bendo)) %>%
  pivot_longer(GCL_perc:total_perc,
               names_to="type",
               values_to="perc") %>%
  separate(type, sep="_", into=c("layer", "protein")) %>%
  dplyr::select(-protein) %>%
  mutate(layer = as.factor(layer),
         mouse = fct_reorder(as.factor(mouse), perc,
                             .fun=max, .desc=FALSE),
         time = as.factor(time)) %>%
  filter(layer != "total")
head(anovpercs, 2)
```

```
## # A tibble: 2 x 7
##   mouse light time  sex     age layer  perc
##   <fct> <fct> <fct> <chr> <dbl> <fct> <dbl>
## 1 11    light day   F        95 GCL    3.14
## 2 11    light day   F        95 INL    7.44
```

Checking Normality

```
#checking if the cell counts are normally distributed
shapiro.test(counts$INL_chat)
```

```
##
##  Shapiro-Wilk normality test
##
## data:  counts$INL_chat
## W = 0.89571, p-value = 0.1396
```

```
shapiro.test(counts$INL_bendo)
```

```
##
##  Shapiro-Wilk normality test
##
## data:  counts$INL_bendo
## W = 0.91934, p-value = 0.2805
```

```
shapiro.test(counts$GCL_chat)
```

```
##
##  Shapiro-Wilk normality test
##
## data:  counts$GCL_chat
## W = 0.88355, p-value = 0.09733
```

```
shapiro.test(counts$GCL_bendo)
```

```
##
##  Shapiro-Wilk normality test
##
## data:  counts$GCL_bendo
## W = 0.86341, p-value = 0.05396
```

```
shapiro.test(counts$total_chat)
```

```
##
##  Shapiro-Wilk normality test
##
## data:  counts$total_chat
## W = 0.88056, p-value = 0.08911
```

```
shapiro.test(counts$total_bendo)
```

```
##
##  Shapiro-Wilk normality test
##
## data:  counts$total_bendo
## W = 0.90417, p-value = 0.1795
```

Checking MANOVA Assumptions

```
manset <- counts %>% dplyr::select(light:time, GCL_perc:INL_perc)

#testing manova assumptions
#multivariate normality
#normal distribution for each combo of indep and dependent variables
anovpercs %>% filter(light == "light" & layer =="GCL") %>% pull(perc) %>% shapiro.test(.)
```

```
##
##  Shapiro-Wilk normality test
##
## data:  .
## W = 0.99193, p-value = 0.9934
```

```
anovpercs %>% filter(light == "dark" & layer =="GCL") %>% pull(perc) %>% shapiro.test(.)
```

```
##
##  Shapiro-Wilk normality test
##
## data:  .
## W = 0.93389, p-value = 0.6104
```

```
anovpercs %>% filter(light == "light" & layer =="INL") %>% pull(perc) %>% shapiro.test(.)
```

```
##
##  Shapiro-Wilk normality test
##
## data:  .
## W = 0.80407, p-value = 0.06392
```

```
anovpercs %>% filter(light == "dark" & layer =="INL") %>% pull(perc) %>% shapiro.test(.)
```

```
##
##  Shapiro-Wilk normality test
##
## data:  .
## W = 0.91084, p-value = 0.442
```

```
anovpercs %>% filter(time == "day" & layer =="GCL") %>% pull(perc) %>% shapiro.test(.)
```

```
##
##  Shapiro-Wilk normality test
##
## data:  .
## W = 0.9171, p-value = 0.4847
```

```
anovpercs %>% filter(time == "night" & layer =="GCL") %>% pull(perc) %>% shapiro.test(.)
```

```
##
##  Shapiro-Wilk normality test
##
## data:  .
## W = 0.96858, p-value = 0.8828
```

```
anovpercs %>% filter(time == "day" & layer =="INL") %>% pull(perc) %>% shapiro.test(.)
```

```
##
##  Shapiro-Wilk normality test
##
## data:  .
## W = 0.86996, p-value = 0.226
```

```
anovpercs %>% filter(time == "night" & layer =="INL") %>% pull(perc) %>% shapiro.test(.)
```

```
##
##  Shapiro-Wilk normality test
##
## data:  .
## W = 0.95699, p-value = 0.7962
```

```
#all pass


anovpercs %>% filter(light == "light" & layer =="GCL") %>% pull(perc) %>% shapiro.test(.)
```

```
##
##  Shapiro-Wilk normality test
##
## data:  .
## W = 0.99193, p-value = 0.9934
```

```
#checking homogenous variance in each combo
#null hyp is that variance is equal
car::leveneTest(manset$GCL_perc, group=manset$light)
```

```
## Levene's Test for Homogeneity of Variance (center = median)
##       Df F value Pr(>F)
## group  1  0.0151 0.9047
##       10
```

```
car::leveneTest(manset$INL_perc, group=manset$light)
```

```
## Levene's Test for Homogeneity of Variance (center = median)
##       Df F value Pr(>F)
## group  1  0.0504 0.8268
##       10
```

```
car::leveneTest(manset$GCL_perc, group=manset$time)
```

```
## Levene's Test for Homogeneity of Variance (center = median)
##       Df F value Pr(>F)
## group  1  2.5789 0.1394
##       10
```

```
car::leveneTest(manset$INL_perc, group=manset$time)
```

```
## Levene's Test for Homogeneity of Variance (center = median)
##       Df F value Pr(>F)
## group  1  1.3768 0.2678
##       10
```

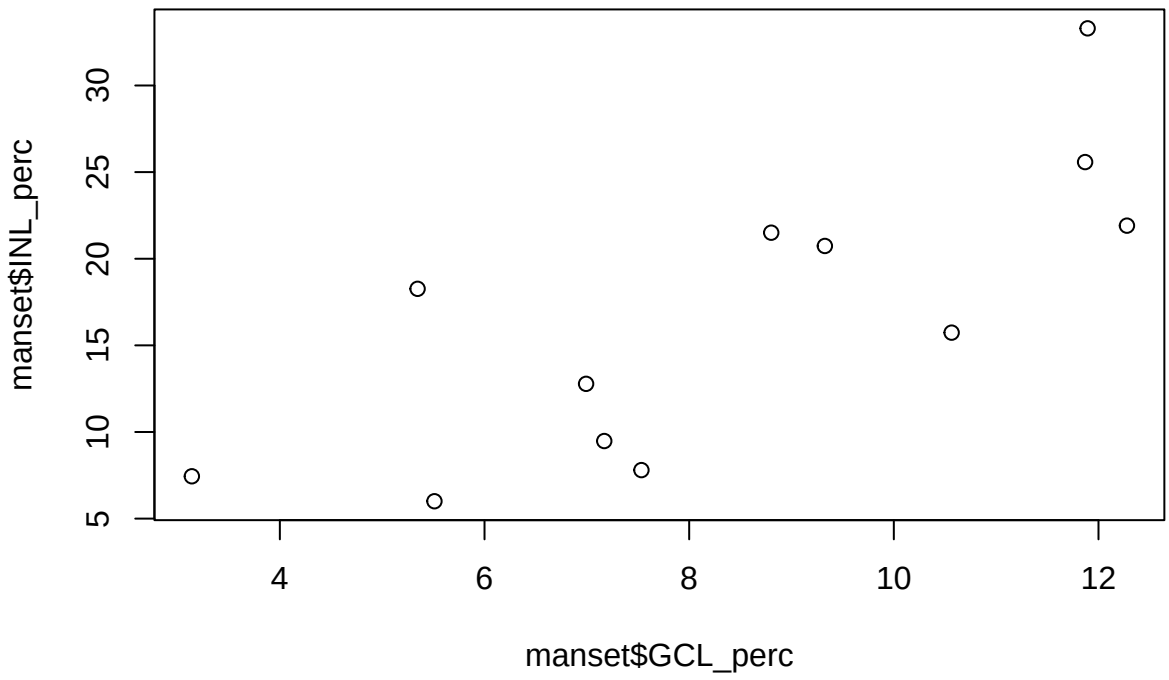
```
#checking for multicollinearity
#low values are good
inllm <- lm(INL_perc ~ light * time, manset)
car::vif(inllm)
```

```
##      light       time light:time
##          2          2          3
```

```
gcllm <- lm(GCL_perc ~ light * time, manset)
car::vif(gcllm)
```

```
##      light       time light:time
##          2          2          3
```

```
#linearity of dep variables
plot(manset$GCL_perc, manset$INL_perc)
```




```
#good to go on manova!
```

MANOVA

```
#manova
mantest <- manova(cbind(GCL_perc, INL_perc) ~ light * time, data=manset)
summary(mantest)
```

```
##            Df  Pillai approx F num Df den Df  Pr(>F)
## light       1 0.37461   2.0965      2      7 0.19343
## time        1 0.11699   0.4637      2      7 0.64697
## light:time  1 0.66218   6.8605      2      7 0.02241 *
## Residuals   8
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
summary.aov(mantest)
```

```
##  Response GCL_perc :
##             Df Sum Sq Mean Sq F value   Pr(>F)
## light        1 10.693  10.693  3.0196 0.120464
## time         1  0.167   0.167  0.0471 0.833664
```

```
## light:time   1 55.374  55.374 15.6365 0.004211 **
## Residuals    8 28.331   3.541
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
##  Response INL_perc :
##             Df Sum Sq Mean Sq F value  Pr(>F)
## light        1 179.82 179.818  4.2818 0.07231 .
## time         1  21.74  21.741  0.5177 0.49231
## light:time   1 233.58 233.584  5.5621 0.04607 *
## Residuals    8 335.96  41.996
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
#light:time interaction is significant overall and for GCL and INL
#look at comparisons through univariate models for each dep variable
summary(gcllm)
```

```
##
## Call:
## lm(formula = GCL_perc ~ light * time, data = manset)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -2.28500 -1.18579  0.03093  0.96322  2.41406
##
## Coefficients:
##                     Estimate Std. Error t value Pr(>|t|)
## (Intercept)            5.395      1.086   4.965  0.00110 **
## lightdark              6.184      1.537   4.025  0.00382 **
## timenight              4.061      1.537   2.643  0.02959 *
## lightdark:timenight   -8.593      2.173  -3.954  0.00421 **
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 1.882 on 8 degrees of freedom
## Multiple R-squared:  0.7004, Adjusted R-squared:  0.5881
## F-statistic: 6.234 on 3 and 8 DF,  p-value: 0.01728
```

```
summary(inllm)
```

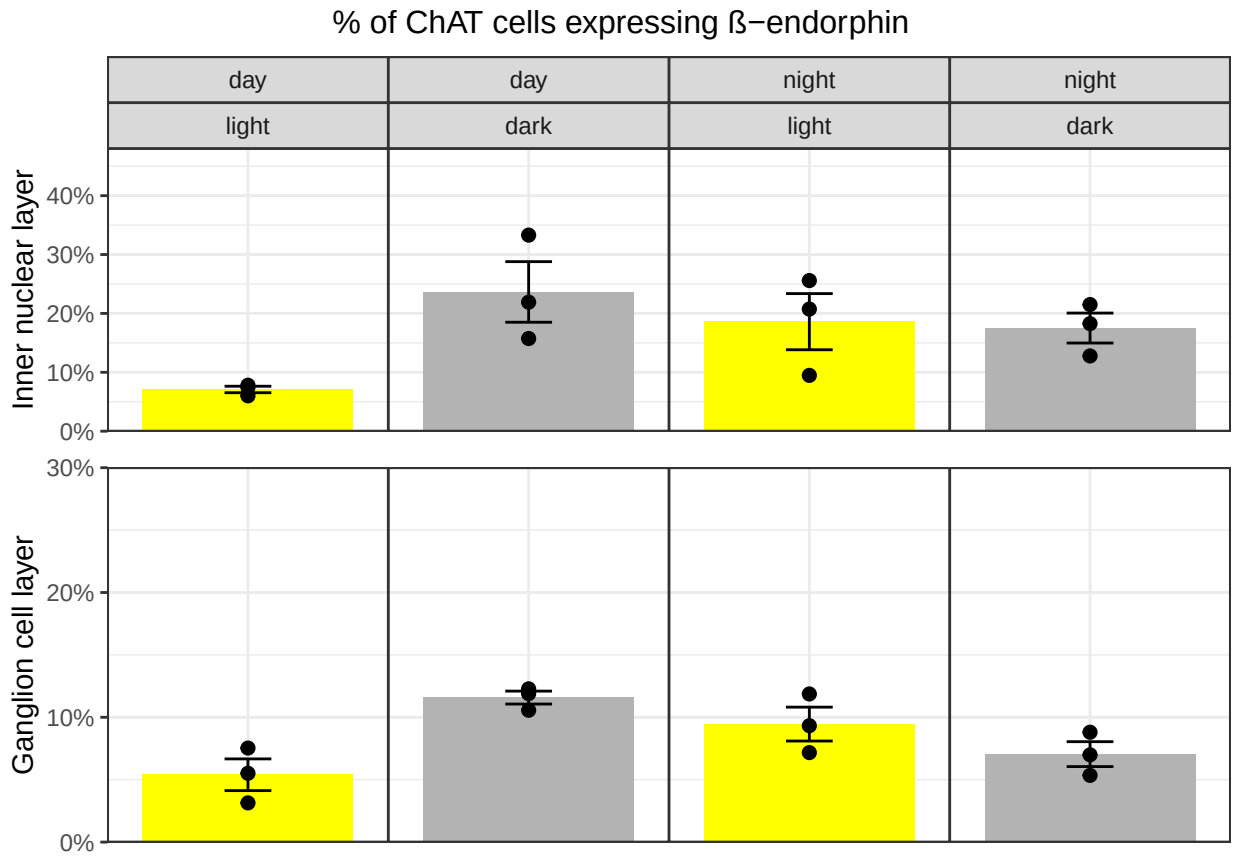
```
##
## Call:
## lm(formula = INL_perc ~ light * time, data = manset)
##
## Residuals:
##     Min      1Q  Median      3Q     Max
## -9.1204 -2.4874  0.5396  2.6011  9.6505
##
## Coefficients:
##                     Estimate Std. Error t value Pr(>|t|)
## (Intercept)            7.082      3.741   1.893   0.0950 .
## lightdark             16.566      5.291   3.131   0.0140 *
```

```
## timenight             11.516      5.291   2.176   0.0612 .
## lightdark:timenight  -17.648      7.483  -2.358   0.0461 *
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 6.48 on 8 degrees of freedom
## Multiple R-squared:  0.5643, Adjusted R-squared:  0.4009
## F-statistic: 3.454 on 3 and 8 DF,  p-value: 0.07147
```

Plotting

```
plotting_manset <- counts %>%
  dplyr::select(mouse,light:time, GCL_perc:INL_perc)


plotting_manset$group <- c("1", "1", "1", "2", "2", "2", "3", "3", "3", "4", "4", "4")


inlpointmanset <- plotting_manset %>%
  mutate(mouse = fct_reorder(as.factor(mouse), INL_perc,
                       .fun=max, .desc=FALSE))

inlpointplot <- inlpointmanset %>% ggplot(aes(x=group, y=INL_perc, fill=light)) +
  stat_summary(fun = 'mean', geom="bar") +
  stat_summary(fun.data = mean_se, geom = "errorbar", width=.2) +
  geom_point(size =2) +
  facet_wrap(time ~ factor(light, c("light", "dark")),
             scales="free_x",
             nrow=1,
             as.table=FALSE) +
  theme_bw() %+replace%
  theme(axis.title.x=element_blank(),
        axis.text.x=element_blank(),
        axis.ticks.x = element_blank(),
        legend.position = "none",
        panel.spacing = unit(0,"pt"),
        plot.background = element_rect(fill = "transparent",colour = NA),
        panel.background = element_rect(fill = "transparent",colour = NA)) +
  labs(y="Inner nuclear layer") +
  scale_y_continuous(labels=function(x) paste0(x,"%"),
                     limits=c(0,40),
                     expand = expansion(mult = c(0, 0.2))) +
  scale_fill_manual(values=c("yellow", "gray70"))


gclpointmanset <- plotting_manset %>%
  mutate(mouse = fct_reorder(as.factor(mouse), GCL_perc,
                       .fun=max, .desc=FALSE))

gclpointplot <- gclpointmanset %>% ggplot(aes(x=group, y=GCL_perc, fill=light)) +
  stat_summary(fun = 'mean', geom="bar") +
  stat_summary(fun.data = mean_se, geom = "errorbar", width=.2) +
```

```
  geom_point(size=2) +
  facet_wrap(time ~ factor(light, c("light", "dark")),
             scales="free_x",
             nrow=1,
             as.table=FALSE) +
  theme_bw() %+replace%
  theme(axis.title.x=element_blank(),
        axis.text.x=element_blank(),
        axis.ticks.x = element_blank(),
        legend.position = "none",
        strip.background = element_blank(),
        strip.text.x = element_blank(),
        panel.spacing = unit(0, "pt"),
        plot.background = element_rect(fill = "transparent",colour = NA),
        panel.background = element_rect(fill = "transparent",colour = NA)) +
  labs(y="Ganglion cell layer") +
  scale_y_continuous(labels=function(x) paste0(x,"%"),
                     limits=c(0,30),
                     expand = expansion(mult = c(0, 0))) +
  scale_fill_manual(values=c("yellow", "gray70"))

bothpointplots <- ggarrange(inlpointplot, gclpointplot, nrow=2)

bothpointplots <- annotate_figure(bothpointplots,
                                  top="% of ChAT cells expressing \U03B2-endorphin")
bothpointplots
```




```
ggsave("../figures/manova_bendo_ihc.png", plot=bothpointplots, width=6, height=6, bg = "transparent")
```

Old ANOVA analysis

```
aov <- aov(perc ~ light * time * layer, anovpercs)
summary(aov)
```

```
##                  Df Sum Sq Mean Sq F value   Pr(>F)
## light             1  139.1   139.1   6.110 0.025056 *
## time              1    9.0     9.0   0.397 0.537298
## layer             1  417.5   417.5  18.337 0.000571 ***
## light:time        1  258.2   258.2  11.341 0.003919 **
## light:layer       1   51.4    51.4   2.258 0.152427
## time:layer        1   12.9    12.9   0.565 0.463282
## light:time:layer  1   30.7    30.7   1.351 0.262231
## Residuals        16  364.3    22.8
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
TukeyHSD(aov)
```

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

```
##     95% family-wise confidence level
##
## Fit: aov(formula = perc ~ light * time * layer, data = anovpercs)
##
## $light
##                diff       lwr      upr    p adj
## dark-light 4.815019 0.6854227 8.944616 0.025056
##
## $time
##               diff       lwr      upr     p adj
## night-day 1.228134 -2.901462 5.357731 0.5372978
##
## $layer
##             diff      lwr      upr     p adj
## INL-GCL 8.341742 4.212145 12.47134 0.0005715
##
## $`light:time`
##                               diff          lwr       upr     p adj
## dark:day-light:day     11.375105   3.49327511 19.256934 0.0039392
## light:night-light:day   7.788220  -0.09360982 15.670049 0.0533578
## dark:night-light:day    6.043154  -1.83867592 13.924983 0.1672187
## light:night-dark:day   -3.586885 -11.46871446  4.294945 0.5748113
## dark:night-dark:day    -5.331951 -13.21378056  2.549879 0.2528778
## dark:night-light:night -1.745066  -9.62689563  6.136763 0.9197070
##
## $`light:layer`
##                            diff        lwr       upr     p adj
## dark:GCL-light:GCL    1.887984 -5.9938453  9.769814 0.9011799
## light:INL-light:GCL   5.414707 -2.4671227 13.296536 0.2414734
## dark:INL-light:GCL   13.156761  5.2749316 21.038591 0.0010656
## light:INL-dark:GCL    3.526723 -4.3551070 11.408552 0.5877993
## dark:INL-dark:GCL    11.268777  3.3869473 19.150606 0.0042612
## dark:INL-light:INL    7.742054 -0.1397752 15.623884 0.0550899
##
## $`time:layer`
##                             diff       lwr       upr     p adj
## night:GCL-day:GCL   -0.2357322 -8.117562  7.646097 0.9997654
## day:INL-day:GCL      6.8778753 -1.003954 14.759705 0.0986522
## night:INL-day:GCL    9.5698762  1.688047 17.451706 0.0149110
## day:INL-night:GCL    7.1136075 -0.768222 14.995437 0.0844234
## night:INL-night:GCL  9.8056084  1.923779 17.687438 0.0125448
## night:INL-day:INL    2.6920009 -5.189829 10.573830 0.7641311
##
## $`light:time:layer`
##                                         diff         lwr       upr     p adj
## dark:day:GCL-light:day:GCL       6.18425591  -7.3043521 19.672864 0.7510493
## light:night:GCL-light:day:GCL    4.06053948  -9.4280686 17.549148 0.9601054
## dark:night:GCL-light:day:GCL     1.65225203 -11.8363560 15.140860 0.9998280
## light:day:INL-light:day:GCL      1.68702656 -11.8015815 15.175635 0.9998025
## dark:day:INL-light:day:GCL      18.25297992   4.7643719 31.741588 0.0047085
## light:night:INL-light:day:GCL   13.20292650  -0.2856816 26.691535 0.0573262
## dark:night:INL-light:day:GCL    12.12108175  -1.3675263 25.609690 0.0950936
## light:night:GCL-dark:day:GCL    -2.12371644 -15.6123245 11.364892 0.9991136
## dark:night:GCL-dark:day:GCL     -4.53200388 -18.0206119  8.956604 0.9313412
```

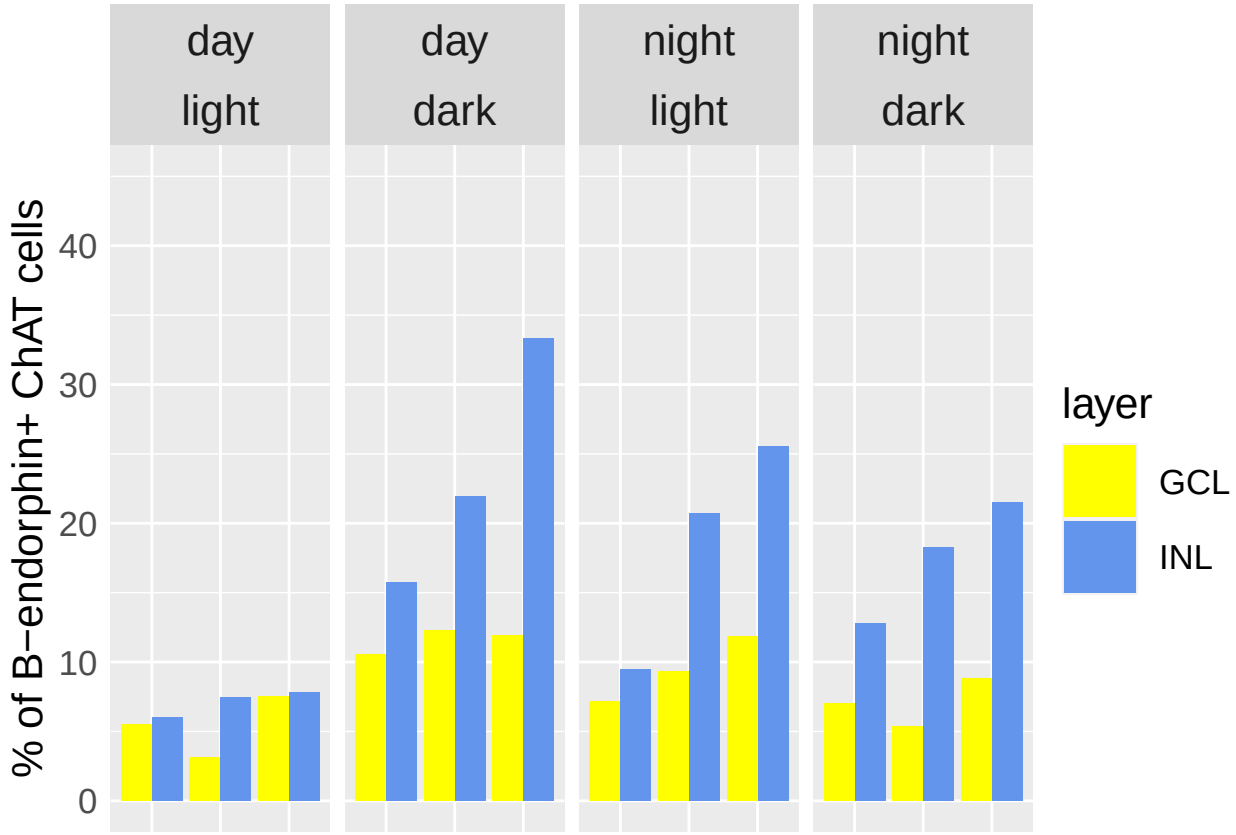
```
## light:day:INL-dark:day:GCL      -4.49722936 -17.9858374  8.991379 0.9338179
## dark:day:INL-dark:day:GCL       12.06872400  -1.4198841 25.557332 0.0973977
## light:night:INL-dark:day:GCL     7.01867059  -6.4699375 20.507279 0.6278714
## dark:night:INL-dark:day:GCL      5.93682583  -7.5517822 19.425434 0.7848034
## dark:night:GCL-light:night:GCL  -2.40828744 -15.8968955 11.080321 0.9980338
## light:day:INL-light:night:GCL   -2.37351292 -15.8621210 11.115095 0.9982051
## dark:day:INL-light:night:GCL    14.19244044   0.7038324 27.681049 0.0355493
## light:night:INL-light:night:GCL  9.14238703  -4.3462210 22.630995 0.3281466
## dark:night:INL-light:night:GCL   8.06054227  -5.4280658 21.549150 0.4713661
## light:day:INL-dark:night:GCL     0.03477453 -13.4538335 13.523383 1.0000000
## dark:day:INL-dark:night:GCL     16.60072789   3.1121198 30.089336 0.0107646
## light:night:INL-dark:night:GCL  11.55067447  -1.9379336 25.039283 0.1230287
## dark:night:INL-dark:night:GCL   10.46882971  -3.0197783 23.957438 0.1959052
## dark:day:INL-light:day:INL      16.56595336   3.0773453 30.054561 0.0109536
## light:night:INL-light:day:INL   11.51589995  -1.9727081 25.004508 0.1249448
## dark:night:INL-light:day:INL    10.43405519  -3.0545529 23.922663 0.1987393
## light:night:INL-dark:day:INL    -5.05005341 -18.5386615  8.438555 0.8875683
## dark:night:INL-dark:day:INL     -6.13189817 -19.6205062  7.356710 0.7583366
## dark:night:INL-light:night:INL  -1.08184476 -14.5704528 12.406763 0.9999901
```

```
#set up a table of p-values for labeling the graphs below
label <- tibble(mouse = c("97", "93", "57"),
                perc = c(Inf, Inf, Inf),
                time = c("day", "night", "night"),
                light = c("dark", "light", "dark"),
                label = c("p=0.00009", "p=0.0058", "p=0.037"))
```

```
#1 row facet wrap, small
bendo_ihc <- anovpercs %>% ggplot(aes(x=mouse, y=perc)) +
  geom_bar(aes(fill=layer), stat="identity", position="dodge") +
  facet_wrap(time ~ factor(light, c("light", "dark")), scales="free_x", nrow=1, as.table=FALSE) +
  labs(y="% of B-endorphin+ ChAT cells",
       col="Light Condition", shape="Cell Layer",
       title = "Figure 2") +
  theme(axis.title.x=element_blank(),
        axis.text.x=element_blank(),
        text = element_text(size=4),
        plot.title = element_text(size=4, margin=margin(0,0,0,0), face = "bold"),
        legend.key.size = unit(0.25, 'cm'),
        strip.text.x = element_text(size = 3, margin = margin(0, 0, 0.02, 0, "cm")),
        axis.ticks = element_blank(),
        legend.margin=margin(0,0,0,0)) +
  scale_fill_manual(values = c("yellow", "cornflowerblue", "grey50")) +
  geom_text(aes(label=label), data=label, vjust="top", hjust="left", size=1)

#ggsave(filename="../figures/bendo_ihc.png", plot=bendo_ihc, height=1, width=2.5)
```

```
#1 row facet wrap, large
bendo_ihc_large <- anovpercs %>% ggplot(aes(x=mouse, y=perc)) +
  geom_bar(aes(fill=layer), stat="identity", position="dodge") +
  facet_wrap(time ~ factor(light, c("light", "dark")), scales="free_x", nrow=1, as.table=FALSE) +
  labs(y="% of B-endorphin+ ChAT cells",
       col="Light Condition", shape="Cell Layer") +
```

```
  theme(axis.title.x=element_blank(),
        axis.text.x=element_blank(),
        text = element_text(size=16),
        legend.key.size = unit(1, 'cm'),
        strip.text.x = element_text(size = 16, margin = margin(0.2, 0, 0.2, 0, "cm")),
        axis.ticks = element_blank(),
        legend.margin=margin(0,0,0,0)) +
  scale_fill_manual(values = c("yellow", "cornflowerblue", "grey50")) +
  expand_limits(y=c(0,45))
  #geom_text(aes(label=label), data=label, vjust="top", hjust="left", size=6)

bendo_ihc_large
```




```
#ggsave(filename="../figures/bendo_ihc_large_nostats.png", plot=bendo_ihc_large, height=5, width=12)
```

```
#2 BY 2 FACET WRAP
bendo_ihc_boxy <- anovpercs %>% ggplot(aes(x=mouse, y=perc)) +
  geom_bar(aes(fill=layer), stat="identity", position="dodge") +
  facet_wrap(time ~ factor(light, c("light", "dark")), scales="free_x", nrow=2, as.table=TRUE) +
  labs(y="% of B-endorphin+ ChAT cells",
       col="Light Condition", shape="Cell Layer") +
  theme(axis.title.x=element_blank(),
        axis.text.x=element_blank(),
        text = element_text(size=4),
        plot.title = element_text(size=4, margin=margin(0,0,0,0), face = "bold"),
```

```
        legend.key.size = unit(0.25, 'cm'),
        strip.text.x = element_text(size = 3, margin = margin(0, 0, 0.02, 0, "cm")),
        axis.ticks = element_blank(),
        legend.margin=margin(0,0,0,0)) +
  scale_fill_manual(values = c("yellow", "cornflowerblue", "grey50")) +
  geom_text(aes(label=label), data=label, vjust="top", hjust="left", size=1)

#ggsave(filename="../figures/bendo_ihc_boxy.png", plot=bendo_ihc_boxy, height=4, width=4)
```