Analysis of Sex Differences in Morphine Deposition in the Plasma, Retina & Brain

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7/12/2022

Reading in & tidying the data

```
sex_diff <- read.csv("../data/morphine_retina_cx_plasma_sex_diff.csv",
                     fileEncoding = 'UTF-8-BOM')
sex_diff$conc_wt <- (sex_diff$raw_conc/sex_diff$tissue_weight)
sex_diff$log_conc_wt <- log(sex_diff$conc_wt)
sex_diff$log_conc_raw <- log(sex_diff$raw_conc)

sex_diff <- sex_diff %>% mutate(tissue = factor(as.factor(tissue),
                                                c("plasma", "retina", "brain")),
                                group = factor(as.factor(group),
                                               c("male", "lowE", "highE")),
                                animal = as.factor(animal))

str(sex_diff)
```

```
## 'data.frame':    111 obs. of  11 variables:
##  $ animal       : Factor w/ 37 levels "11F","12F","13F",..: 10 20 27 28 30 32 34 36 37 9 ...
##  $ raw_conc     : num  56.6 22.1 36.8 49.5 87.4 ...
##  $ tissue_weight: num  20.1 10.3 13.7 15.1 31.4 14.7 14.3 20.8 15.7 20.5 ...
##  $ body_wt      : num  27.2 30.1 28.7 25.8 26.1 27.3 26.2 23.7 27.2 22 ...
##  $ dose         : num  20.6 18.6 19.5 21.7 20 19.1 19.9 22 19.2 19.1 ...
##  $ stage        : chr  "male" "male" "male" "male" ...
##  $ group        : Factor w/ 3 levels "male","lowE",..: 1 1 1 1 1 1 1 1 1 3 ...
##  $ tissue       : Factor w/ 3 levels "plasma","retina",..: 3 3 3 3 3 3 3 3 3 3 ...
##  $ conc_wt      : num  2.82 2.14 2.68 3.28 2.78 ...
##  $ log_conc_wt  : num  1.035 0.763 0.987 1.188 1.024 ...
##  $ log_conc_raw : num  4.04 3.09 3.6 3.9 4.47 ...
```

```
#test for outliers
test_out <- rosnerTest(sex_diff$conc_wt,
  k = 4
)
```

```
## Warning in rosnerTest(sex_diff$conc_wt, k = 4): 37 observations with NA/NaN/Inf
## in 'x' removed.
```

```
test_out
```

```
## $distribution
## [1] "Normal"
##
## $statistic
##      R.1      R.2      R.3      R.4
## 2.398976 2.438932 2.194539 2.214792
##
## $sample.size
## [1] 74
##
## $parameters
## k
## 4
##
## $alpha
## [1] 0.05
##
## $crit.value
## lambda.1 lambda.2 lambda.3 lambda.4
## 3.277970 3.273006 3.267957 3.262821
##
## $n.outliers
## [1] 0
##
## $alternative
## [1] "Up to 4 observations are not\n                                    from the same Distribution."
##
## $method
## [1] "Rosner's Test for Outliers"
##
## $data
##  [1]  2.815398  2.143670  2.683080  3.278901  2.783952  2.489524  2.914196
##  [8]  3.023846  2.264051  2.126278  2.158567  1.099614  2.612317  2.321724
## [15]  2.150787  5.069100  1.959265  7.416833  1.840316  2.184907  1.887165
## [22]  2.675675  1.079808  3.696573  2.867145  1.989755  3.406589  2.709242
## [29]  2.220611  2.375481  3.545806  2.180149  2.090560  3.005871  2.745674
## [36]  2.489692  2.099267 27.561395 17.246940 24.693975 22.080050 23.070419
## [43] 13.959157 19.209173 20.999789 13.090263 16.161962 11.789479  7.405556
## [50] 17.296135 14.268936 15.960467 19.374612 15.713000 26.403452 23.226366
## [57] 25.703574 19.560540 17.330184 11.049851 31.412579 22.184283 15.172353
## [64] 22.980760 17.255130 15.395250 18.666395 34.880556 21.168358 22.425167
## [71] 35.646000 25.001300 20.076692 30.730114
##
## $data.name
## [1] "sex_diff$conc_wt"
##
## $bad.obs
## [1] 37
##
## $all.stats
##   i   Mean.i      SD.i    Value Obs.Num    R.i+1 lambda.i+1 Outlier
```

```
## 1 0 11.54799 10.045123 35.64600      71 2.398976   3.277970   FALSE
## 2 1 11.21788  9.702061 34.88056      68 2.438932   3.273006   FALSE
## 3 2 10.88924  9.352005 31.41258      61 2.194539   3.267957   FALSE
## 4 3 10.60018  9.088863 30.73011      74 2.214792   3.262821   FALSE
##
## attr(,"class")
## [1] "gofOutlier"
```

```
SumStat_tissue <- dplyr::summarise(group_by(sex_diff, tissue),
  n = n(),
  mean_tissue_wt = mean(tissue_weight),
  sd_tissue_wt = sd(tissue_weight),
  se_tissue_wt = sd_tissue_wt/sqrt(n),
)
SumStat_tissue
```

```
## # A tibble: 3 x 5
##   tissue     n mean_tissue_wt sd_tissue_wt se_tissue_wt
##   <fct>  <int>          <dbl>        <dbl>        <dbl>
## 1 plasma    37          NA          NA           NA
## 2 retina    37           4.76        0.739        0.122
## 3 brain     37          17.4         8.77         1.44
```

```
SumStat_all <- dplyr::summarise(group_by(sex_diff, tissue),
  n = n(),
  mean_conc_raw = mean(raw_conc),
  sd_conc_raw = sd(raw_conc),
  se_conc_raw = sd_conc_raw/sqrt(n),
  mean_conc_raw_log = mean(log(raw_conc)),
  sd_conc_raw_log = sd(log(raw_conc)),
  se_conc_raw_log = sd_conc_raw_log/sqrt(n),
  mean_conc_wt = mean(conc_wt),
  sd_conc_wt = sd(conc_wt),
  se_conc_wt = sd_conc_wt/sqrt(n),
  log_conc = mean(log(conc_wt)),
  sd_conc_log = sd(log(conc_wt)),
  se_conc_log = sd_conc_log/sqrt(n),
  mean_wt = mean(body_wt),
  sd_wt = sd(body_wt),
  mean_dose = mean(dose),
  sd_dose = sd(dose),
)

SumStat_all
```

```
## # A tibble: 3 x 18
##   tissue     n mean_co~1 sd_co~2 se_co~3 mean_~4 sd_co~5 se_co~6 mean_~7 sd_co~8
##   <fct>  <int>     <dbl>   <dbl>   <dbl>   <dbl>   <dbl>   <dbl>   <dbl>   <dbl>
## 1 plasma    37     342.     73.3   12.1     5.81   0.274  0.0450   NA      NA
## 2 retina    37      94.5    22.2    3.65    4.52   0.265  0.0436   20.4     6.41
## 3 brain     37      43.2    22.2    3.64    3.65   0.475  0.0780    2.66    1.07
## # ... with 8 more variables: se_conc_wt <dbl>, log_conc <dbl>,
## #   sd_conc_log <dbl>, se_conc_log <dbl>, mean_wt <dbl>, sd_wt <dbl>,
```

```
## #   mean_dose <dbl>, sd_dose <dbl>, and abbreviated variable names
## #   1: mean_conc_raw, 2: sd_conc_raw, 3: se_conc_raw, 4: mean_conc_raw_log,
## #   5: sd_conc_raw_log, 6: se_conc_raw_log, 7: mean_conc_wt, 8: sd_conc_wt
```

```
SumStat_sexdiff <- dplyr::summarise(group_by(sex_diff, tissue, group),
  n = n(),
  mean_conc_raw = mean(raw_conc),
  sd_conc_raw = sd(raw_conc),
  se_conc_raw = sd_conc_raw/sqrt(n),
  mean_conc_raw_log = mean(log(raw_conc)),
  sd_conc_raw_log = sd(log(raw_conc)),
  se_conc_raw_log = sd_conc_raw_log/sqrt(n),
  mean_conc_wt = mean(conc_wt),
  sd_conc_wt = sd(conc_wt),
  se_conc_wt = sd_conc_wt/sqrt(n),
  log_conc = mean(log(conc_wt)),
  sd_conc_log = sd(log(conc_wt)),
  se_conc_log = sd_conc_log/sqrt(n),
  mean_wt = mean(body_wt),
  sd_wt = sd(body_wt),
  mean_dose = mean(dose),
  sd_dose = sd(dose),
)
```

```
## `summarise()` has grouped output by 'tissue'. You can override using the
## `.groups` argument.
```

```
SumStat_sexdiff
```

```
## # A tibble: 9 x 19
## # Groups:   tissue [3]
##   tissue group     n mean_conc~1 sd_co~2 se_co~3 mean_~4 sd_co~5 se_co~6 mean_~7
##   <fct>  <fct> <int>       <dbl>   <dbl>   <dbl>   <dbl>   <dbl>   <dbl>   <dbl>
## 1 plasma male      9       346.     63.2   21.1     5.83   0.170  0.0567   NA
## 2 plasma lowE      8       331.     59.5   21.0     5.79   0.176  0.0621   NA
## 3 plasma highE    20       345.     84.4   18.9     5.80   0.343  0.0767   NA
## 4 retina male      9        96.6    12.7    4.22    4.56   0.133  0.0444   20.2
## 5 retina lowE      8        95.6    20.9    7.39    4.54   0.243  0.0860   20.6
## 6 retina highE    20        93.1    26.4    5.91    4.49   0.320  0.0715   20.5
## 7 brain  male      9        47.7    19.3    6.43    3.79   0.398  0.133     2.71
## 8 brain  lowE      8        46.4    23.5    8.30    3.73   0.488  0.173     2.53
## 9 brain  highE    20        40.0    23.4    5.22    3.56   0.501  0.112     2.69
## # ... with 9 more variables: sd_conc_wt <dbl>, se_conc_wt <dbl>,
## #   log_conc <dbl>, sd_conc_log <dbl>, se_conc_log <dbl>, mean_wt <dbl>,
## #   sd_wt <dbl>, mean_dose <dbl>, sd_dose <dbl>, and abbreviated variable names
## #   1: mean_conc_raw, 2: sd_conc_raw, 3: se_conc_raw, 4: mean_conc_raw_log,
## #   5: sd_conc_raw_log, 6: se_conc_raw_log, 7: mean_conc_wt
```

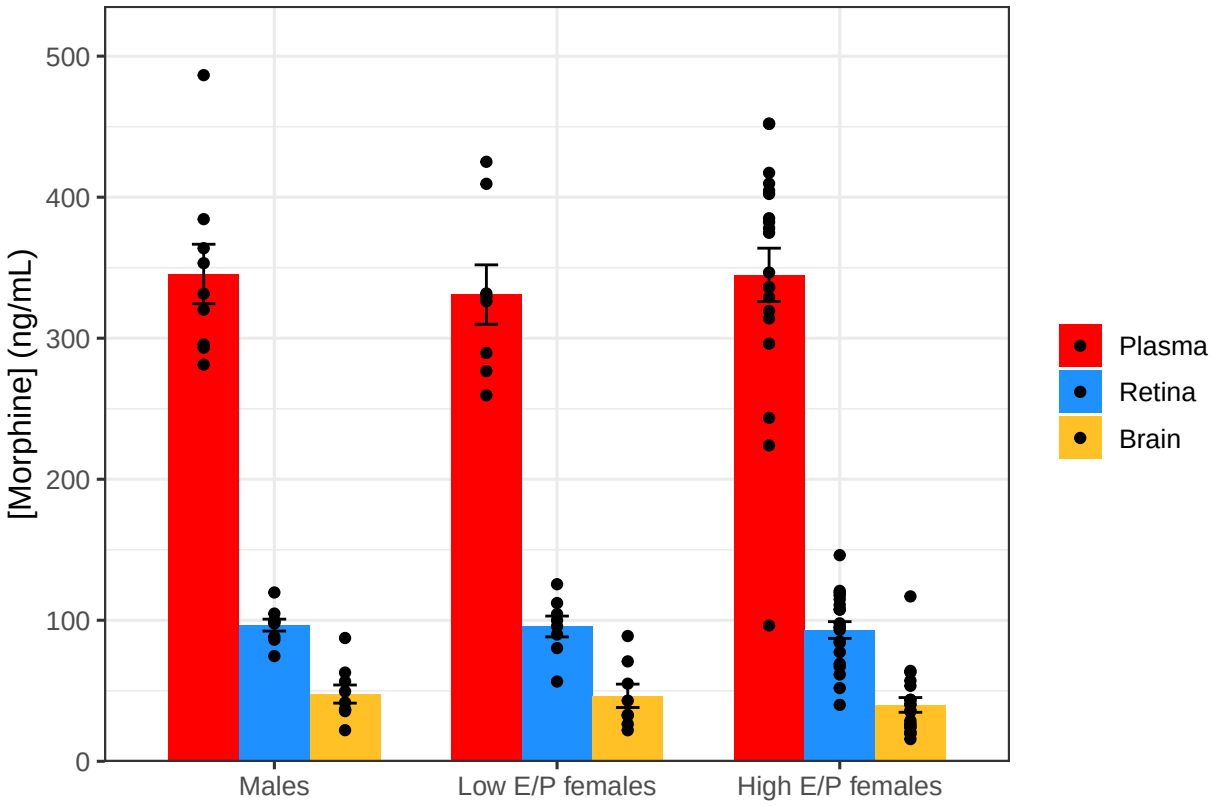
Brain, Retina & Plasma - raw concentrations

Visualizations for potential sex and tissue-dependent differences

```
plotall_tissue <- ggplot() +
  geom_bar(data=SumStat_sexdiff, aes(x=group, y=mean_conc_raw, fill=tissue),
           stat="identity", position=position_dodge(width=0.75), width=0.75) +
  geom_errorbar(data=SumStat_sexdiff, aes(x=group, ymin=mean_conc_raw - se_conc_raw,
                                          ymax=mean_conc_raw + se_conc_raw, fill=tissue),
                position=position_dodge(width=0.75), width=.25) +
  geom_point(data=sex_diff, aes(x=group, y=raw_conc, fill=tissue),
             position=position_dodge(width=0.75)) +
  scale_y_continuous(name="[Morphine] (ng/mL)",
                     expand = expansion(mult = c(0, 0.1))) +
  theme_bw(base_size = 12) %+replace%
    theme(legend.title = element_blank()) +
  scale_fill_manual(values=c("red", "dodgerblue", "goldenrod1"),
                    labels=c("plasma" = "Plasma", "retina" = "Retina",
                             "brain" = "Brain")) +
  scale_x_discrete(labels=c("male" = "Males", "lowE" = "Low E/P females",
                            "highE" = "High E/P females")) +
  xlab("")
```

```
## Warning in geom_errorbar(data = SumStat_sexdiff, aes(x = group, ymin =
## mean_conc_raw - : Ignoring unknown aesthetics: fill
```

```
plotall_tissue
```

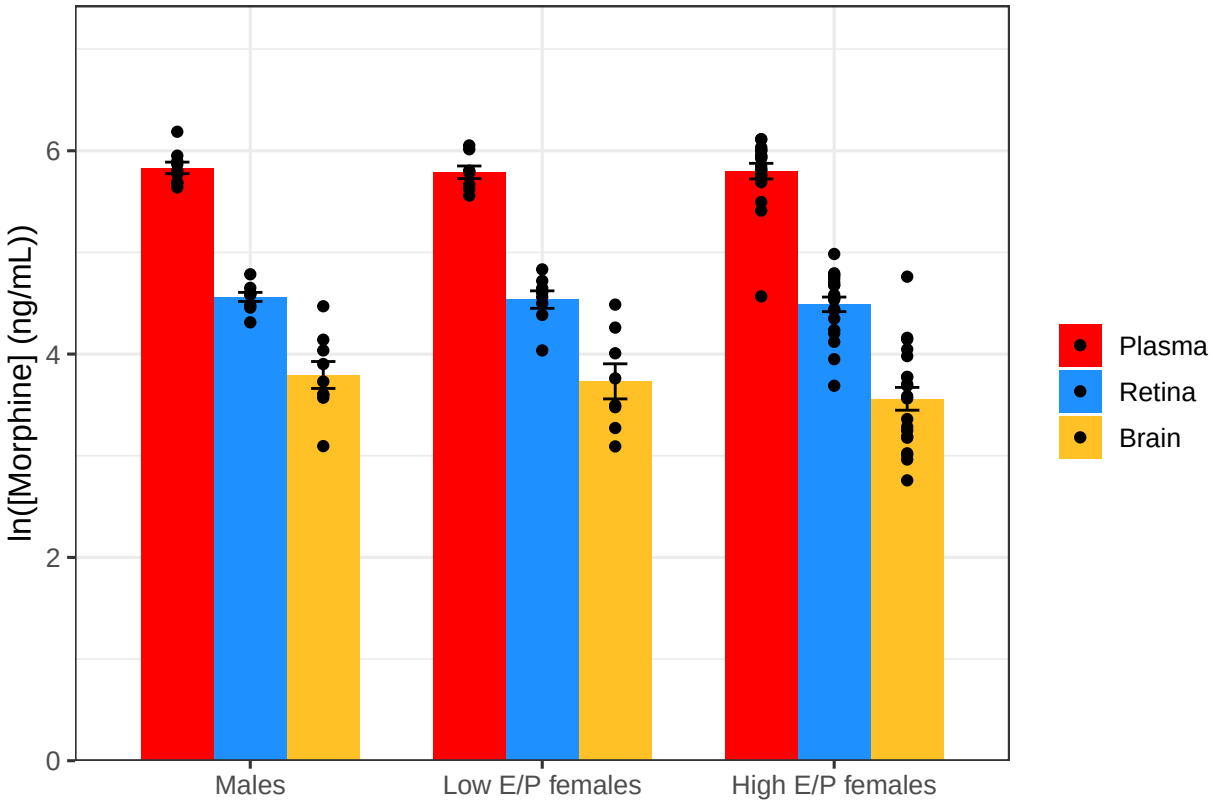


```
#ggsave("../figures/conc_all_tissues.png", plot=plotall_tissue, width=6, height=4)
#ggsave("../figures/conc_all_tissues.svg", plot=plotall_tissue, width=6, height=4)

plotall_tissueL <- ggplot() +
  geom_bar(data=SumStat_sexdiff, aes(x=group, y=mean_conc_raw_log, fill=tissue),
           stat="identity", position=position_dodge(width=0.75), width=0.75) +
  geom_errorbar(data=SumStat_sexdiff, aes(x=group,ymin=mean_conc_raw_log - se_conc_raw_log,
                                          ymax=mean_conc_raw_log + se_conc_raw_log, fill=tissue),
                position=position_dodge(width=0.75), width=.25) +
  geom_point(data=sex_diff, aes(x=group, y=log_conc_raw, fill=tissue),
             position=position_dodge(width=0.75)) +
  scale_y_continuous(name="ln([Morphine] (ng/mL))",
                     expand = expansion(mult = c(0, 0.2))) +
  theme_bw(base_size = 12) %+replace%
    theme(legend.title = element_blank()) +
  scale_fill_manual(values=c("red", "dodgerblue", "goldenrod1"),
                    labels=c("plasma" = "Plasma", "retina" = "Retina",
                             "brain" = "Brain")) +
  scale_x_discrete(labels=c("male" = "Males", "lowE" = "Low E/P females",
                            "highE" = "High E/P females")) +
  xlab("")
```

```
## Warning in geom_errorbar(data = SumStat_sexdiff, aes(x = group, ymin =
## mean_conc_raw_log - : Ignoring unknown aesthetics: fill
```

```
plotall_tissueL
```

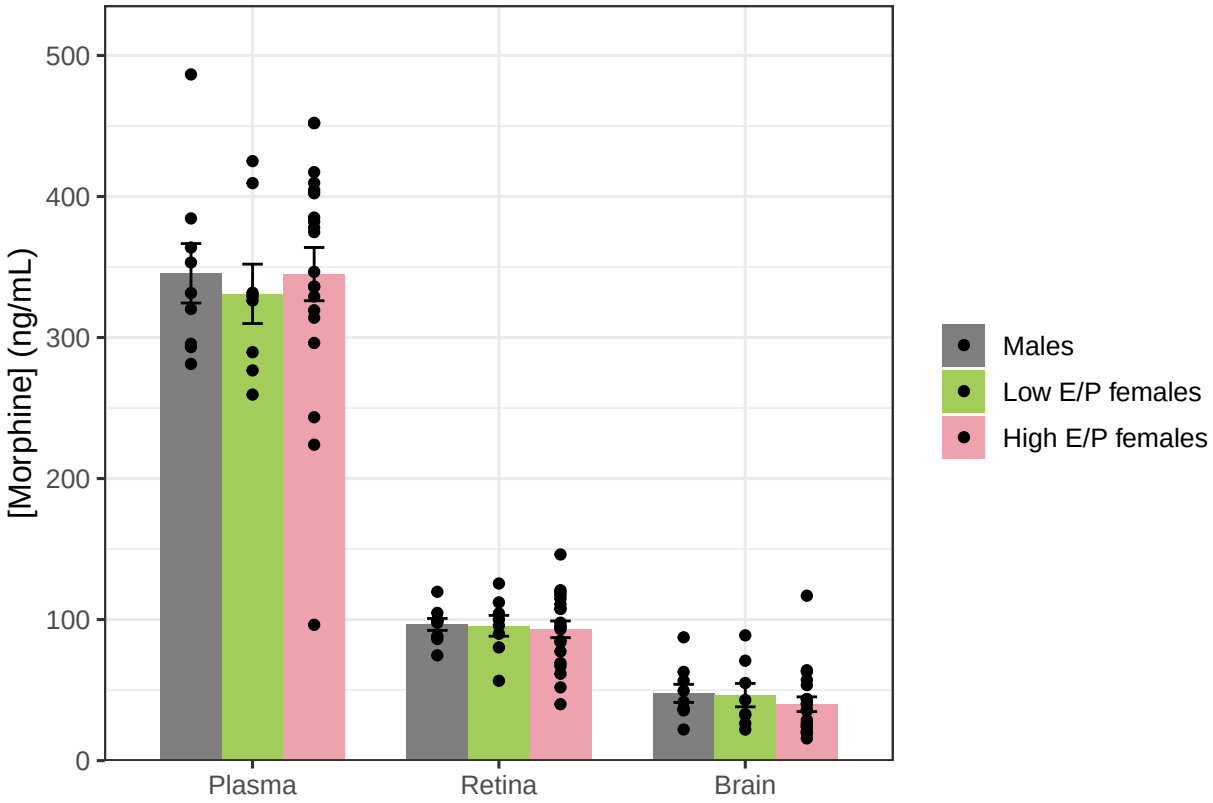



```
#ggsave("../figures/conc_all_tissues_log.png", plot=plotall_tissueL, width=6, height=4)
#ggsave("../figures/conc_all_tissues_log.svg", plot=plotall_tissueL, width=6, height=4)

plotall_stage <- ggplot() +
  geom_bar(data=SumStat_sexdiff, aes(x=tissue, y=mean_conc_raw, fill=group),
           stat="identity", position=position_dodge(width=0.75), width=0.75) +
  geom_errorbar(data=SumStat_sexdiff, aes(x=tissue,ymin=mean_conc_raw - se_conc_raw,
                                          ymax=mean_conc_raw + se_conc_raw, fill=group),
                position=position_dodge(width=0.75), width=.25) +
  geom_point(data=sex_diff, aes(x=tissue, y=raw_conc, fill=group),
             position=position_dodge(width=0.75)) +
  scale_y_continuous(name="[Morphine] (ng/mL)",
                     expand = expansion(mult = c(0, 0.1))) +
  theme_bw(base_size = 12) %+replace%
    theme(legend.title = element_blank()) +
  scale_fill_manual(labels=c("male" = "Males", "lowE" = "Low E/P females",
                             "highE" = "High E/P females"),
                    values=c("gray50", "darkolivegreen3", "lightpink2")) +
  scale_x_discrete(labels=c("plasma" = "Plasma", "retina" = "Retina",
                            "brain" = "Brain")) +
  xlab("")
```

```
## Warning in geom_errorbar(data = SumStat_sexdiff, aes(x = tissue, ymin =
## mean_conc_raw - : Ignoring unknown aesthetics: fill
```

```
plotall_stage
```



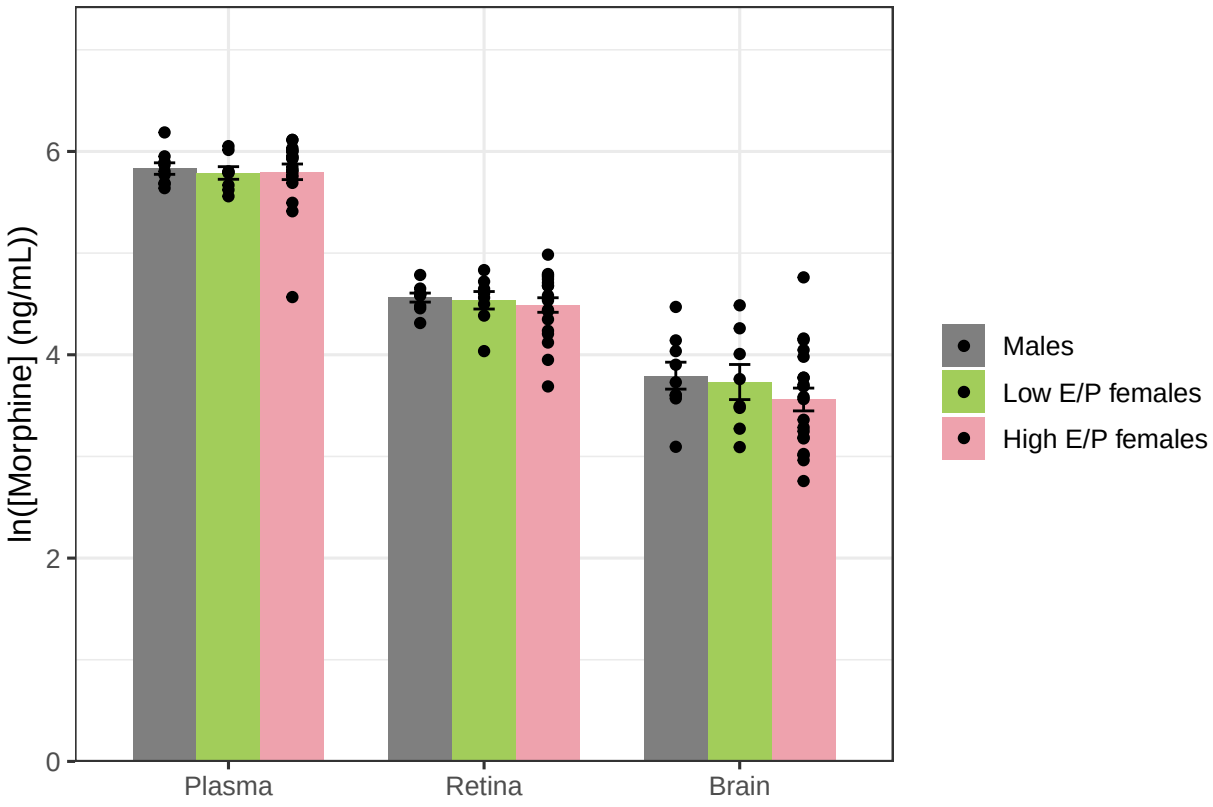

```
ggsave("../figures/conc_all_stage.png", plot=plotall_stage, width=6, height=4)
ggsave("../figures/conc_all_stage.svg", plot=plotall_stage, width=6, height=4)

plotall_stageL <- ggplot() +
  geom_bar(data=SumStat_sexdiff, aes(x=tissue, y=mean_conc_raw_log, fill=group),
           stat="identity", position=position_dodge(width=0.75), width=0.75) +
  geom_errorbar(data=SumStat_sexdiff, aes(x=tissue,ymin=mean_conc_raw_log - se_conc_raw_log,
                                          ymax=mean_conc_raw_log + se_conc_raw_log, fill=group),
                position=position_dodge(width=0.75), width=.25) +
  geom_point(data=sex_diff, aes(x=tissue, y=log_conc_raw, fill=group),
             position=position_dodge(width=0.75)) +
  scale_y_continuous(name="ln([Morphine] (ng/mL))",
                     expand = expansion(mult = c(0, 0.2))) +
  theme_bw(base_size = 12) %+replace%
    theme(legend.title = element_blank()) +
  scale_fill_manual(labels=c("male" = "Males", "lowE" = "Low E/P females",
                             "highE" = "High E/P females"),
                    values=c("gray50", "darkolivegreen3", "lightpink2")) +
  scale_x_discrete(labels=c("plasma" = "Plasma", "retina" = "Retina",
                            "brain" = "Brain")) +
  xlab("")
```

```
## Warning in geom_errorbar(data = SumStat_sexdiff, aes(x = tissue, ymin =
```

```
## mean_conc_raw_log - : Ignoring unknown aesthetics: fill
```

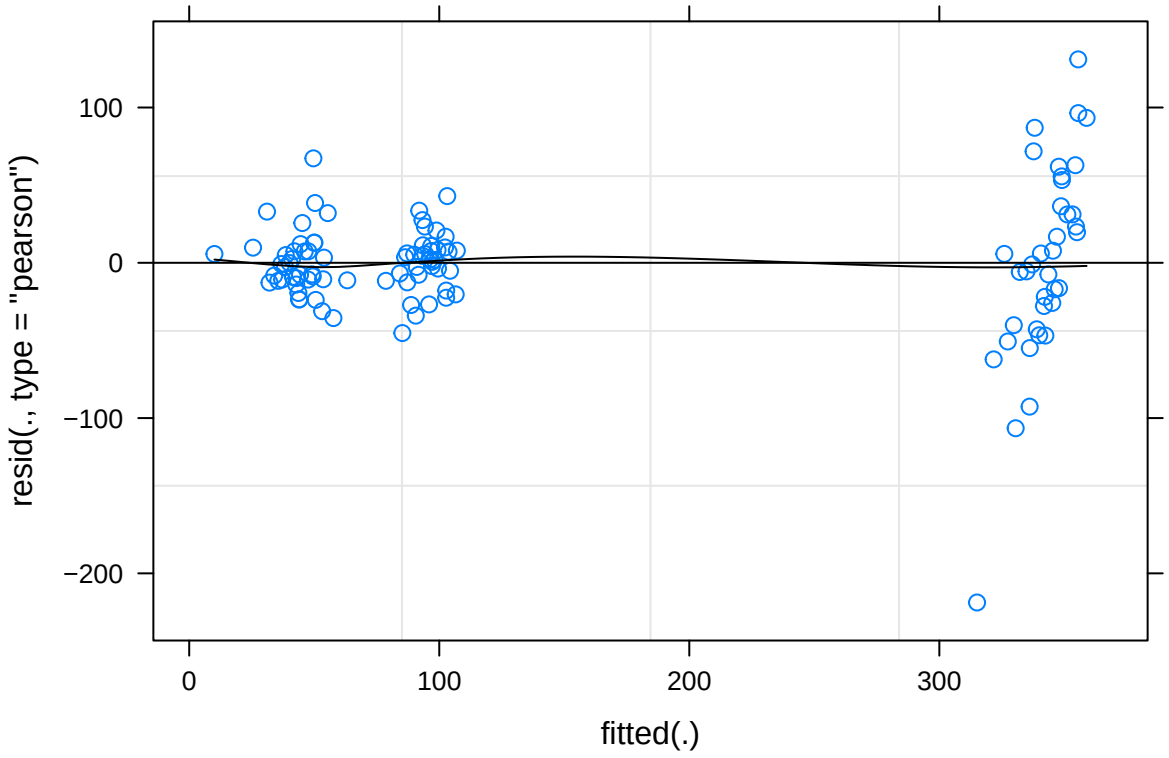
```
plotall_stageL
```



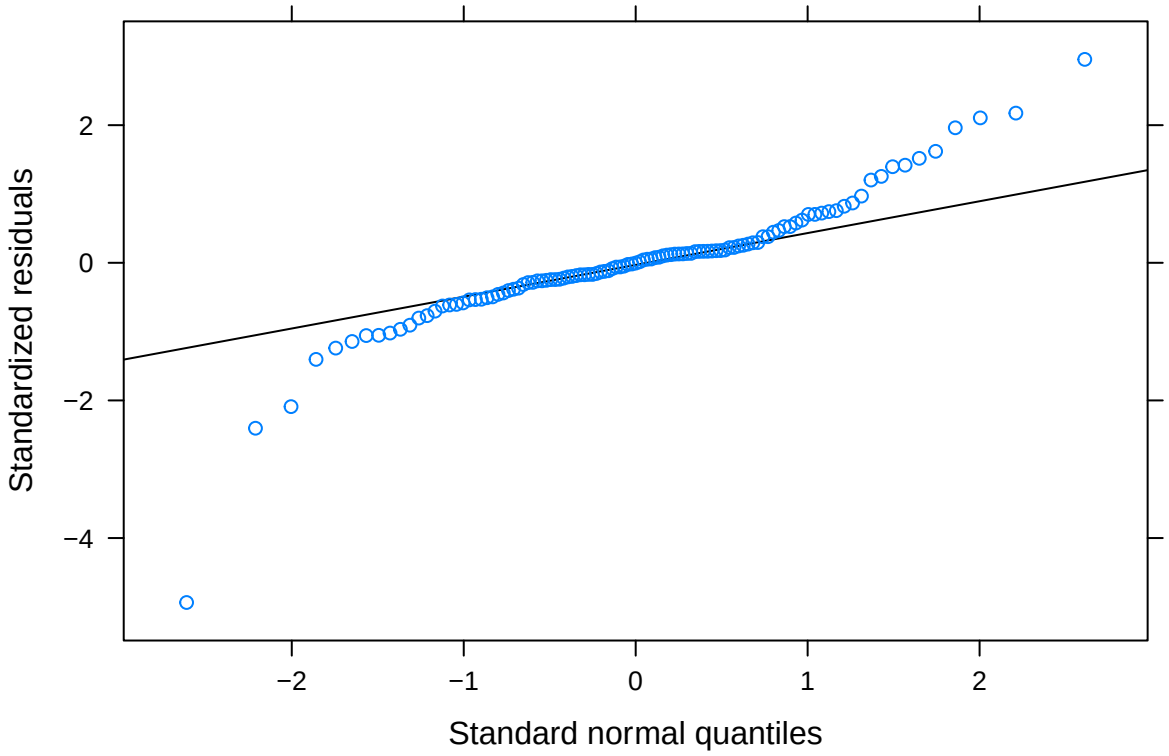

```
#ggsave("../figures/conc_all_stage_log.png", plot=plotall_stageL, width=6, height=4)
#ggsave("../figures/conc_all_stage_log.svg", plot=plotall_stageL, width=6, height=4)
```

Statistical analysis

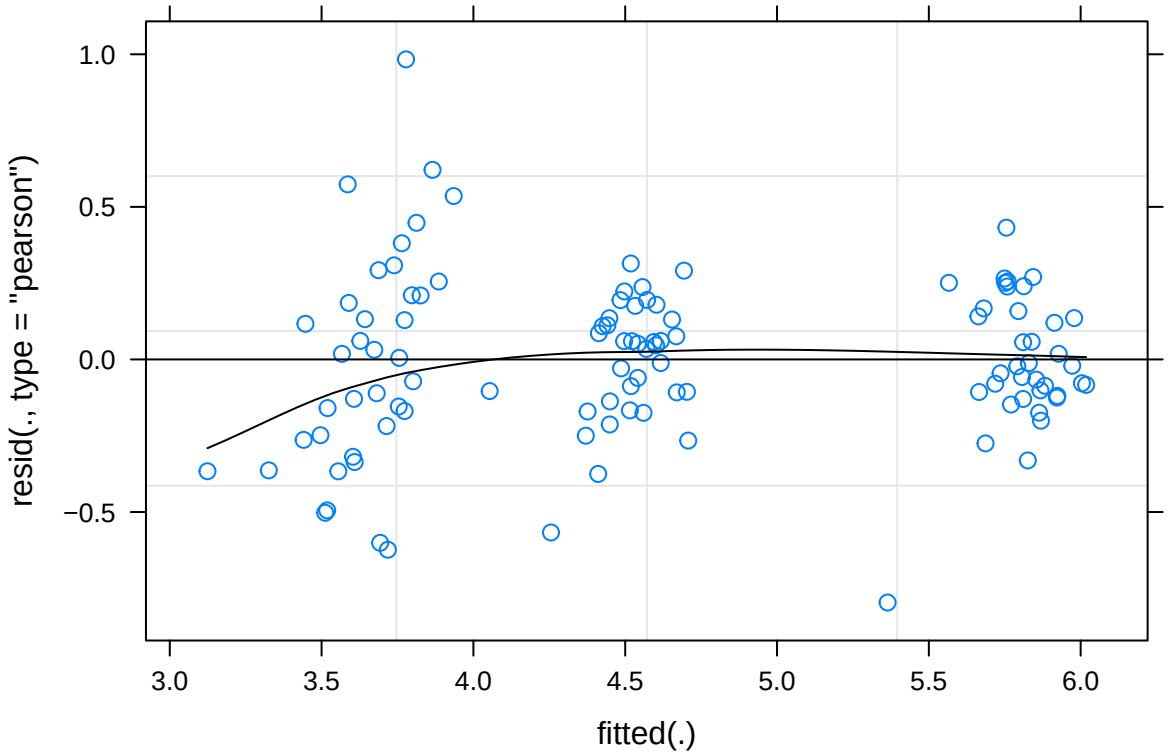
```
lmer_diff_all <- lmer(raw_conc ~ group*tissue+(1|animal), data =sex_diff)
plot(lmer_diff_all, type=c("p","smooth"), col.line=1)
```



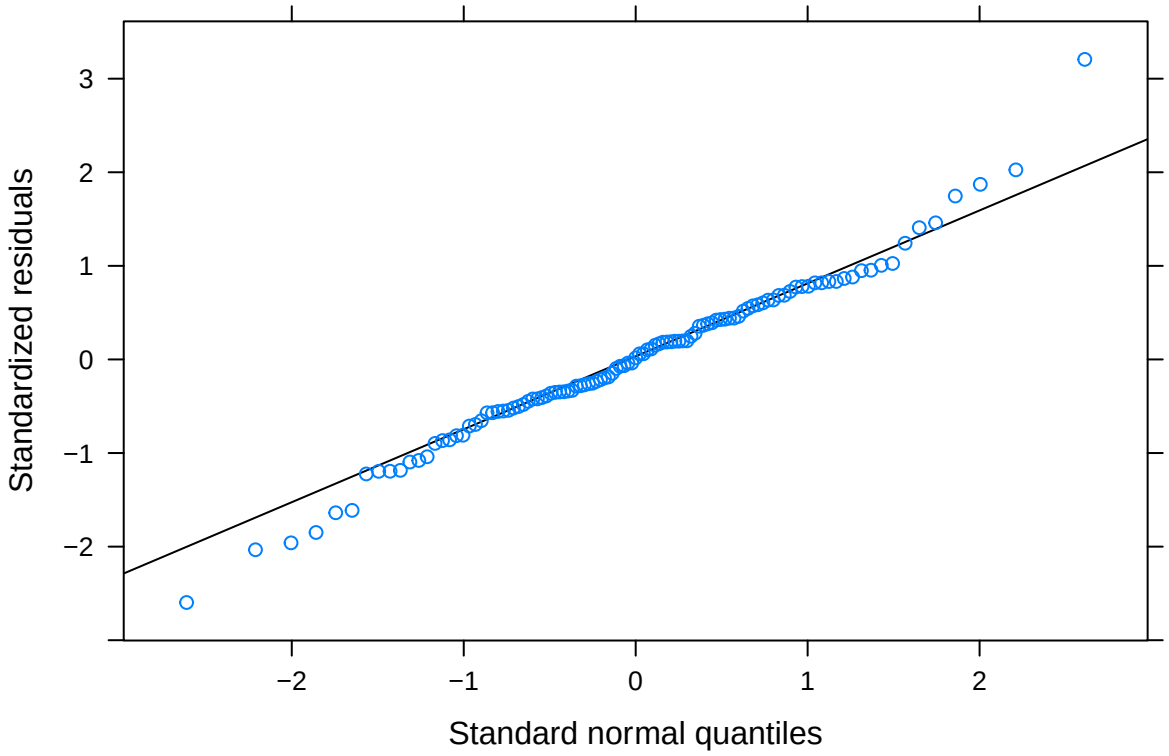

```
lattice::qqmath(lmer_diff_all)
```




```
lmer_diff_all_log <- lmer(log(raw_conc) ~ group*tissue+(1|animal), data =sex_diff)
plot(lmer_diff_all_log, type=c("p","smooth"), col.line=1)
```




```
lattice::qqmath(lmer_diff_all_log)
```




```
anova(lmer_diff_all_log)
```

```
## Type III Analysis of Variance Table with Satterthwaite's method
##              Sum Sq Mean Sq NumDF DenDF  F value Pr(>F)
## group         0.128   0.064     2    34   0.6799 0.5134
## tissue       71.137  35.568     2    68 378.1322 <2e-16 ***
## group:tissue  0.189   0.047     4    68   0.5017 0.7345
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
emmeans::emmeans(lmer_diff_all_log, pairwise ~ tissue| group)$contrasts
```

```
## group = male:
##  contrast        estimate    SE df t.ratio p.value
##  plasma - retina    1.269 0.145 68   8.778  <.0001
##  plasma - brain     2.037 0.145 68  14.091  <.0001
##  retina - brain     0.768 0.145 68   5.313  <.0001
##
## group = lowE:
##  contrast        estimate    SE df t.ratio p.value
##  plasma - retina    1.252 0.153 68   8.166  <.0001
##  plasma - brain     2.057 0.153 68  13.411  <.0001
##  retina - brain     0.804 0.153 68   5.245  <.0001
##
```

```
## group = highE:
##  contrast        estimate    SE df t.ratio p.value
##  plasma - retina    1.311 0.097 68  13.513  <.0001
##  plasma - brain     2.240 0.097 68  23.095  <.0001
##  retina - brain     0.929 0.097 68   9.583  <.0001
##
## Degrees-of-freedom method: kenward-roger
## Results are given on the log (not the response) scale.
## P value adjustment: tukey method for comparing a family of 3 estimates
```

```
emmeans::emmeans(lmer_diff_all_log, pairwise ~ group| tissue)$contrasts
```

```
## tissue = plasma:
##  contrast     estimate    SE   df t.ratio p.value
##  male - lowE    0.0433 0.173 90.2   0.251  0.9660
##  male - highE   0.0318 0.143 90.2   0.222  0.9731
##  lowE - highE  -0.0116 0.149 90.2  -0.078  0.9967
##
## tissue = retina:
##  contrast     estimate    SE   df t.ratio p.value
##  male - lowE    0.0265 0.173 90.2   0.153  0.9872
##  male - highE   0.0732 0.143 90.2   0.513  0.8653
##  lowE - highE   0.0467 0.149 90.2   0.314  0.9471
##
## tissue = brain:
##  contrast     estimate    SE   df t.ratio p.value
##  male - lowE    0.0627 0.173 90.2   0.363  0.9301
##  male - highE   0.2345 0.143 90.2   1.643  0.2331
##  lowE - highE   0.1718 0.149 90.2   1.155  0.4831
##
## Degrees-of-freedom method: kenward-roger
## Results are given on the log (not the response) scale.
## P value adjustment: tukey method for comparing a family of 3 estimates
```

```
#shapiro.test(resid(lmer_diff))
```

Brain & Retina normalized by tissue weight

```
sex_diff <- sex_diff %>% filter(tissue != "plasma")
SumStat_sexdiff <- SumStat_sexdiff %>% filter(tissue != "plasma")
SumStat_sexdiff
```

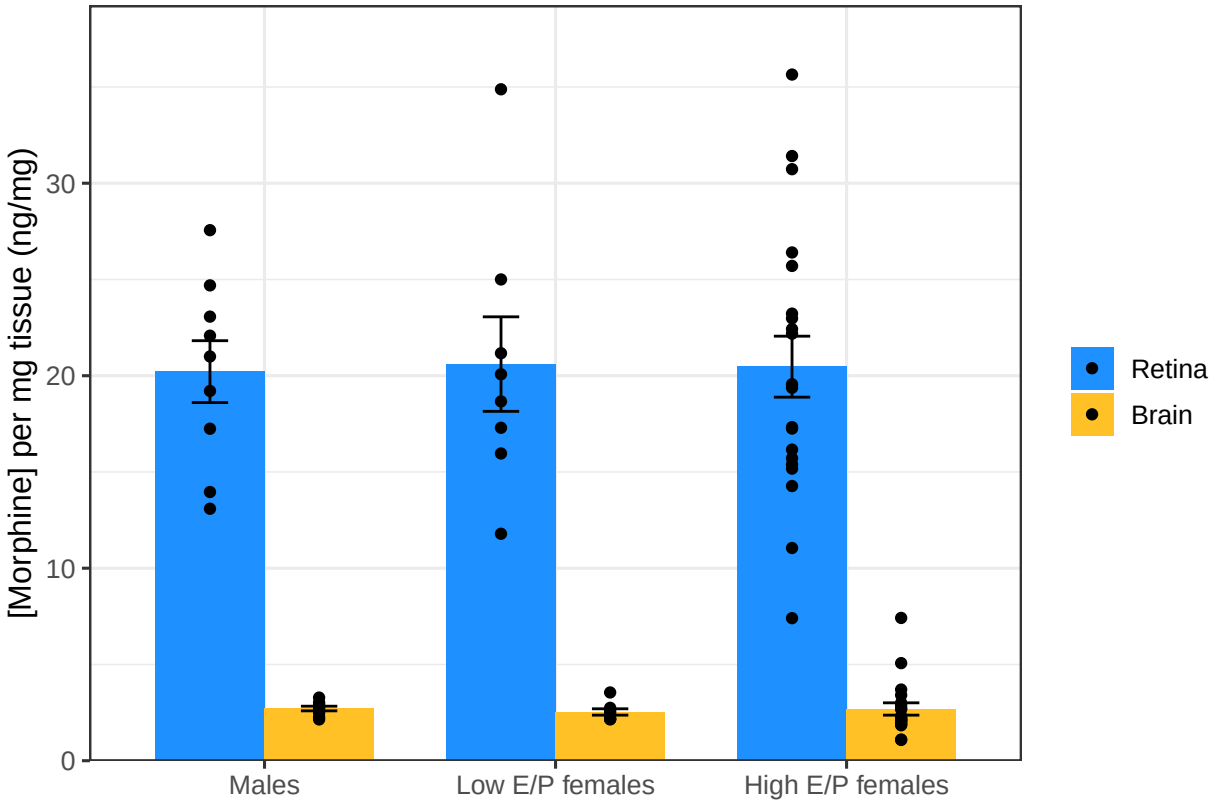
```
## # A tibble: 6 x 19
## # Groups:   tissue [2]
##   tissue group     n mean_conc~1 sd_co~2 se_co~3 mean_~4 sd_co~5 se_co~6 mean_~7
##   <fct>  <fct> <int>       <dbl>   <dbl>   <dbl>   <dbl>   <dbl>   <dbl>   <dbl>
## 1 retina male      9        96.6    12.7    4.22    4.56   0.133  0.0444   20.2
## 2 retina lowE      8        95.6    20.9    7.39    4.54   0.243  0.0860   20.6
## 3 retina highE    20        93.1    26.4    5.91    4.49   0.320  0.0715   20.5
## 4 brain  male      9        47.7    19.3    6.43    3.79   0.398  0.133     2.71
```

```
## 5 brain  lowE      8        46.4    23.5    8.30    3.73   0.488  0.173     2.53
## 6 brain  highE    20        40.0    23.4    5.22    3.56   0.501  0.112     2.69
## # ... with 9 more variables: sd_conc_wt <dbl>, se_conc_wt <dbl>,
## #   log_conc <dbl>, sd_conc_log <dbl>, se_conc_log <dbl>, mean_wt <dbl>,
## #   sd_wt <dbl>, mean_dose <dbl>, sd_dose <dbl>, and abbreviated variable names
## #   1: mean_conc_raw, 2: sd_conc_raw, 3: se_conc_raw, 4: mean_conc_raw_log,
## #   5: sd_conc_raw_log, 6: se_conc_raw_log, 7: mean_conc_wt
```

```
plot_tissue <- ggplot() +
  geom_bar(data=SumStat_sexdiff, aes(x=group, y=mean_conc_wt, fill=tissue),
           stat="identity", position=position_dodge(width=0.75), width=0.75) +
  geom_errorbar(data=SumStat_sexdiff, aes(x=group, ymin=mean_conc_wt - se_conc_wt,
                                          ymax=mean_conc_wt + se_conc_wt, fill=tissue),
                position=position_dodge(width=0.75), width=.25) +
  geom_point(data=sex_diff, aes(x=group, y=conc_wt, fill=tissue),
             position=position_dodge(width=0.75)) +
  scale_y_continuous(name="[Morphine] per mg tissue (ng/mg)",
                     expand = expansion(mult = c(0, 0.1))) +
  theme_bw(12) %+replace%
    theme(legend.title = element_blank()) +
  scale_fill_manual(values=c("dodgerblue", "goldenrod1"),
                    labels=c("retina" = "Retina", "brain" = "Brain")) +
  scale_x_discrete(labels=c("male" = "Males", "lowE" = "Low E/P females",
                            "highE" = "High E/P females")) +
  xlab("")
```

```
## Warning in geom_errorbar(data = SumStat_sexdiff, aes(x = group, ymin =
## mean_conc_wt - : Ignoring unknown aesthetics: fill
```

```
plot_tissue
```

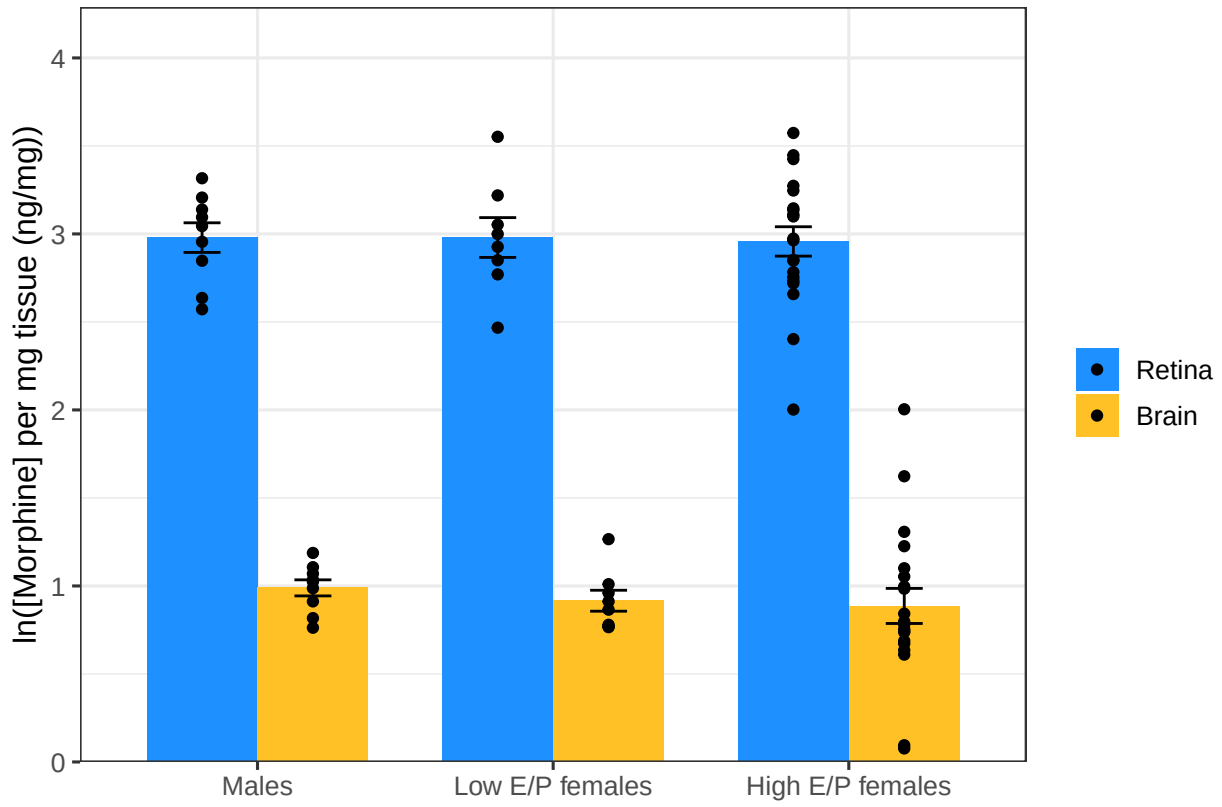



```
#ggsave("../figures/conc_tissue.png", plot=plot_tissue, width=6, height=4)
#ggsave("../figures/conc_tissue.svg", plot=plot_tissue, width=6, height=4)

plot_tissueL <- ggplot() +
  geom_bar(data=SumStat_sexdiff, aes(x=group, y=log_conc, fill=tissue),
           stat="identity", position=position_dodge(width=0.75), width=0.75) +
  geom_errorbar(data=SumStat_sexdiff, aes(x=group,ymin=log_conc - se_conc_log,
                                          ymax=log_conc + se_conc_log, fill=tissue),
                position=position_dodge(width=0.75), width=.25) +
  geom_point(data=sex_diff, aes(x=group, y=log_conc_wt, fill=tissue),
             position=position_dodge(width=0.75)) +
  scale_y_continuous(name="ln([Morphine] per mg tissue (ng/mg))",
                     expand = expansion(mult = c(0, 0.2))) +
  theme_bw(12) %+replace%
    theme(legend.title = element_blank()) +
  scale_fill_manual(values=c("dodgerblue", "goldenrod1"),
                    labels=c("retina" = "Retina", "brain" = "Brain")) +
  scale_x_discrete(labels=c("male" = "Males", "lowE" = "Low E/P females",
                            "highE" = "High E/P females")) +
  xlab("")
```

```
## Warning in geom_errorbar(data = SumStat_sexdiff, aes(x = group, ymin = log_conc
## - : Ignoring unknown aesthetics: fill
```

```
plot_tissueL
```

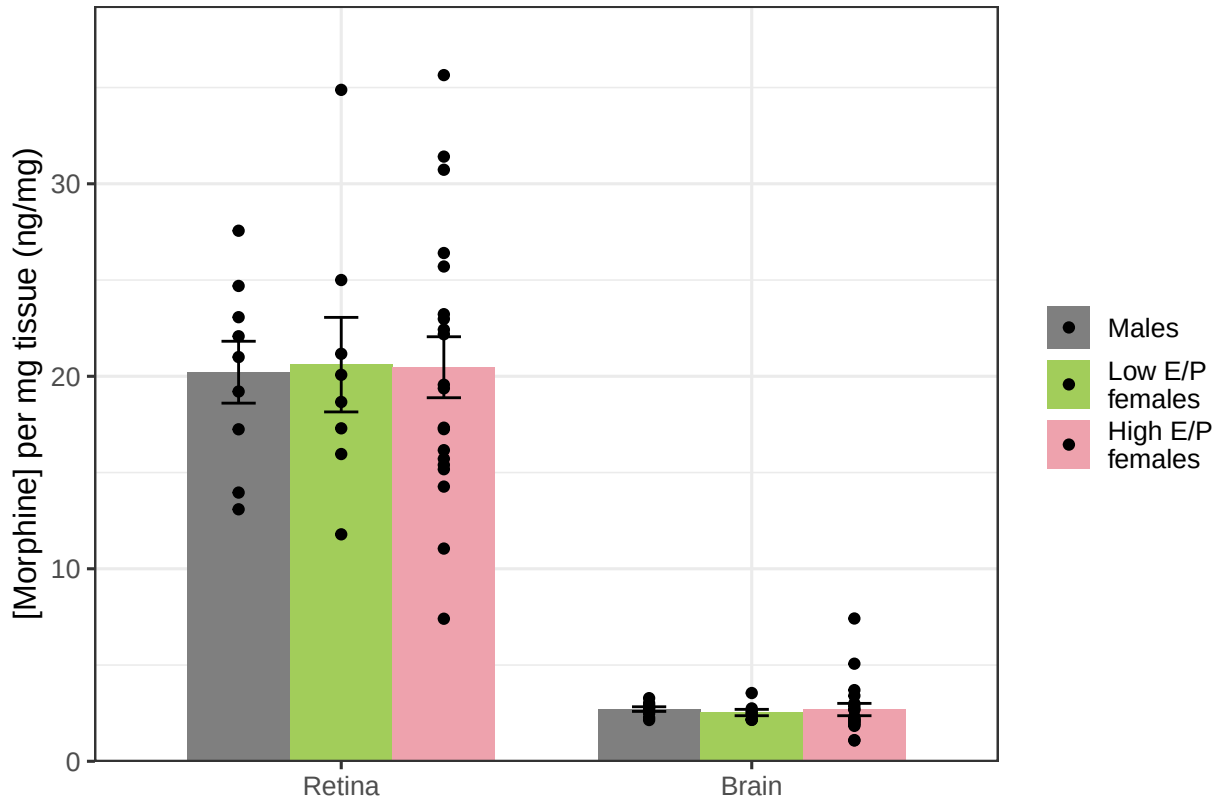



```
ggsave("../figures/conc_tissue_log.png", plot=plot_tissueL, width=6, height=4)
ggsave("../figures/conc_tissue_log.svg", plot=plot_tissueL, width=6, height=4)

plot_stage <- ggplot() +
  geom_bar(data=SumStat_sexdiff, aes(x=tissue, y=mean_conc_wt, fill=group),
           stat="identity", position=position_dodge(width=0.75), width=0.75) +
  geom_errorbar(data=SumStat_sexdiff, aes(x=tissue,ymin=mean_conc_wt - se_conc_wt,
                                          ymax=mean_conc_wt + se_conc_wt, fill=group),
                position=position_dodge(width=0.75), width=.25) +
  geom_point(data=sex_diff, aes(x=tissue, y=conc_wt, fill=group),
             position=position_dodge(width=0.75)) +
  scale_y_continuous(name="[Morphine] per mg tissue (ng/mg)",
                     expand = expansion(mult = c(0, 0.1))) +
  theme_bw(base_size = 12) %+replace%
    theme(legend.title = element_blank(),
          legend.spacing.y = unit(0.1, 'cm')) +
  ## next line needed to make previous line work
  guides(fill = guide_legend(byrow = TRUE)) +
  scale_fill_manual(values=c("gray50", "darkolivegreen3", "lightpink2"),
                    labels=c("male" = "Males", "lowE" = "Low E/P\nfemales",
                             "highE" = "High E/P\nfemales")) +
  scale_x_discrete(labels=c("retina" = "Retina", "brain" = "Brain")) +
  xlab("")
```

```
## Warning in geom_errorbar(data = SumStat_sexdiff, aes(x = tissue, ymin =
## mean_conc_wt - : Ignoring unknown aesthetics: fill
```

```
plot_stage
```



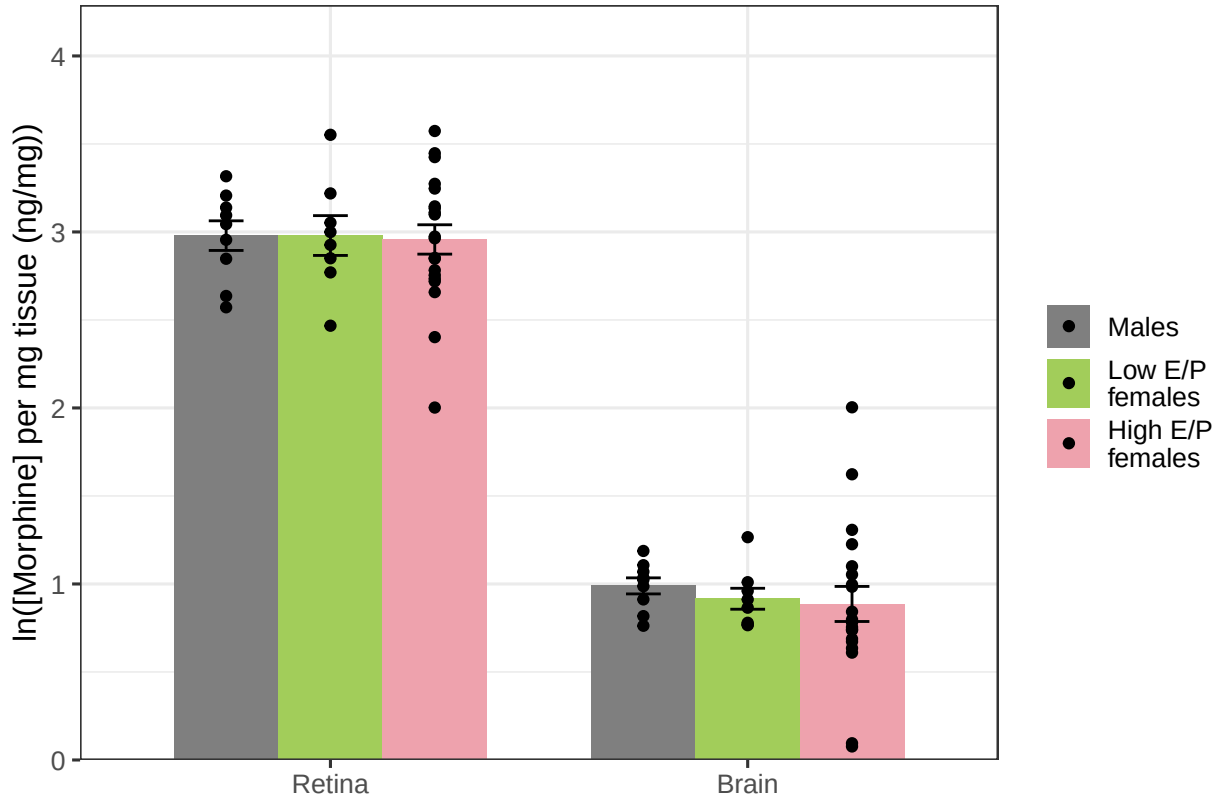

```
#ggsave("../figures/conc_stage.png", plot=plot_stage, width=6, height=4)
#ggsave("../figures/conc_stage.svg", plot=plot_stage, width=6, height=4)


plot_stageL <- ggplot() +
  geom_bar(data=SumStat_sexdiff, aes(x=tissue, y=log_conc, fill=group),
           stat="identity", position=position_dodge(width=0.75), width=0.75) +
  geom_errorbar(data=SumStat_sexdiff, aes(x=tissue,ymin=log_conc - se_conc_log,
                                          ymax=log_conc + se_conc_log, fill=group),
                position=position_dodge(width=0.75), width=.25) +
  geom_point(data=sex_diff, aes(x=tissue, y=log_conc_wt, fill=group),
             position=position_dodge(width=0.75)) +
  scale_y_continuous(name="ln([Morphine] per mg tissue (ng/mg))",
                     expand = expansion(mult = c(0, 0.2))) +
  theme_bw(base_size = 12) %+replace%
    theme(legend.title = element_blank(),
          legend.spacing.y = unit(0.1, 'cm')) +
  ## next line needed to make previous line work
  guides(fill = guide_legend(byrow = TRUE)) +
  scale_fill_manual(values=c("gray50", "darkolivegreen3", "lightpink2"),
```

```
                    labels=c("male" = "Males", "lowE" = "Low E/P\nfemales",
                             "highE" = "High E/P\nfemales")) +
  scale_x_discrete(labels=c("retina" = "Retina", "brain" = "Brain")) +
  xlab("")
```

```
## Warning in geom_errorbar(data = SumStat_sexdiff, aes(x = tissue, ymin = log_conc
## - : Ignoring unknown aesthetics: fill
```

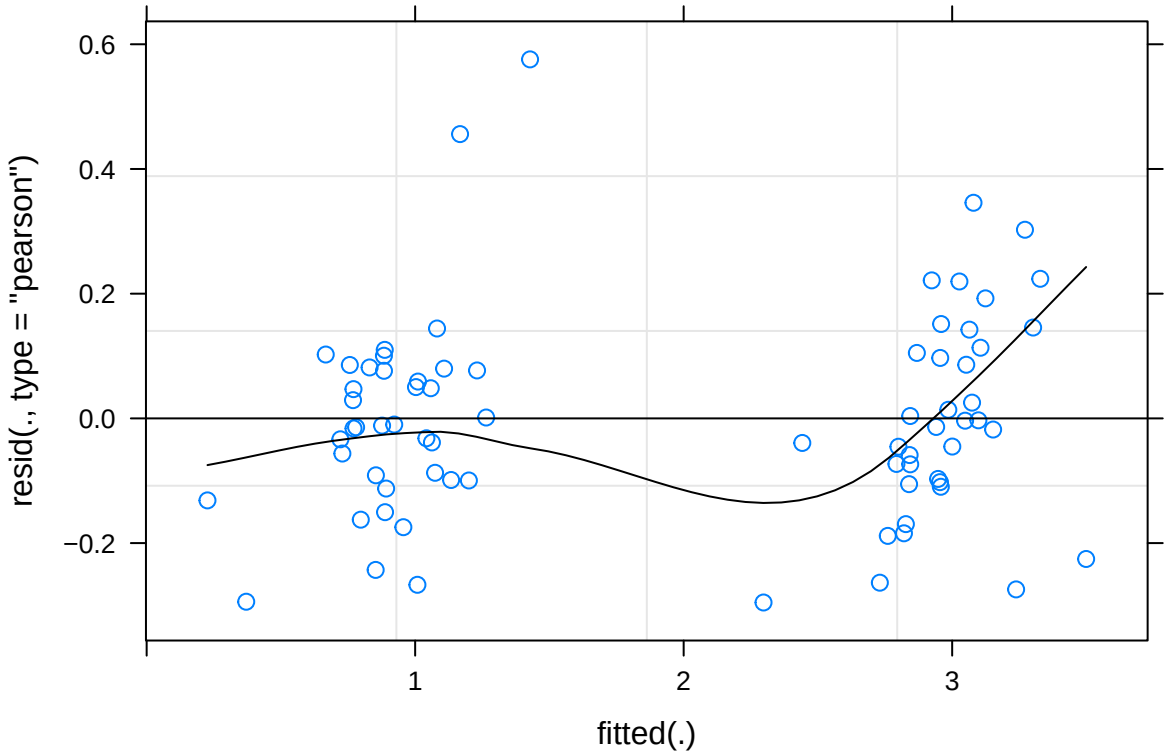
```
plot_stageL
```



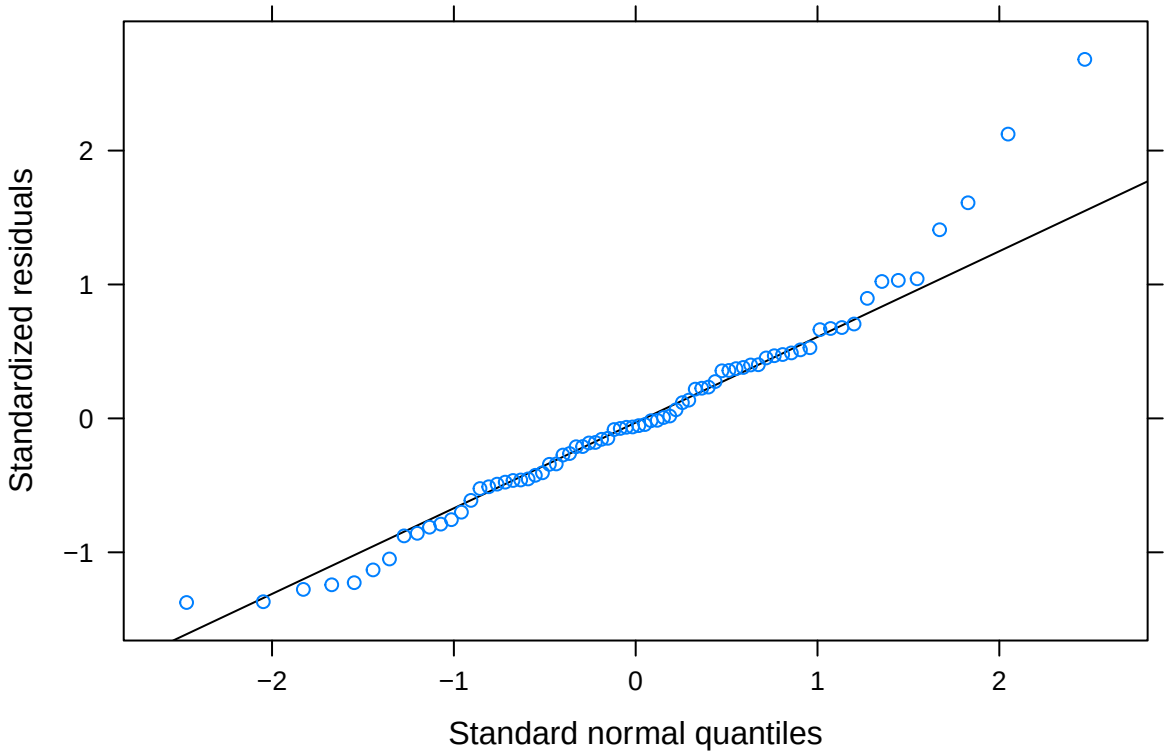

```
#ggsave("../figures/conc_stage_log.png", plot=plot_stageL, width=6, height=4)
#ggsave("../figures/conc_stage_log.svg", plot=plot_stageL, width=6, height=4)
```

Statstical analysis for differences between groups and tissues

```
lmer_diff <- lmer(log(conc_wt) ~ group*tissue+(1|animal), data =sex_diff)
plot(lmer_diff, type=c("p","smooth"), col.line=1)
```




```
lattice::qqmath(lmer_diff)
```




```
anova(lmer_diff)
```

```
## Type III Analysis of Variance Table with Satterthwaite's method
##              Sum Sq Mean Sq NumDF DenDF   F value Pr(>F)
## group         0.012   0.006     2    34    0.1287 0.8796
## tissue       65.555  65.555     1    34 1421.3554 <2e-16 ***
## group:tissue  0.021   0.011     2    34    0.2313 0.7948
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
emmeans::emmeans(lmer_diff, pairwise ~ tissue| group)$contrasts
```

```
## group = male:
##  contrast       estimate     SE df t.ratio p.value
##  retina - brain     1.99 0.1012 34  19.657  <.0001
##
## group = lowE:
##  contrast       estimate     SE df t.ratio p.value
##  retina - brain     2.06 0.1074 34  19.218  <.0001
##
## group = highE:
##  contrast       estimate     SE df t.ratio p.value
##  retina - brain     2.07 0.0679 34  30.495  <.0001
##
## Degrees-of-freedom method: kenward-roger
```

```
## Results are given on the log (not the response) scale.
```

```
emmeans::emmeans(lmer_diff, pairwise ~ group| tissue)$contrasts
```

```
## tissue = retina:
##  contrast     estimate    SE   df t.ratio p.value
##  male - lowE   -0.0006 0.167 49.7  -0.004  1.0000
##  male - highE   0.0217 0.138 49.7   0.158  0.9863
##  lowE - highE   0.0223 0.143 49.7   0.156  0.9867
##
## tissue = brain:
##  contrast     estimate    SE   df t.ratio p.value
##  male - lowE    0.0730 0.167 49.7   0.439  0.8996
##  male - highE   0.1027 0.138 49.7   0.746  0.7371
##  lowE - highE   0.0296 0.143 49.7   0.207  0.9767
##
## Degrees-of-freedom method: kenward-roger
## Results are given on the log (not the response) scale.
## P value adjustment: tukey method for comparing a family of 3 estimates
```

```
#shapiro.test(resid(lmer_diff))
```

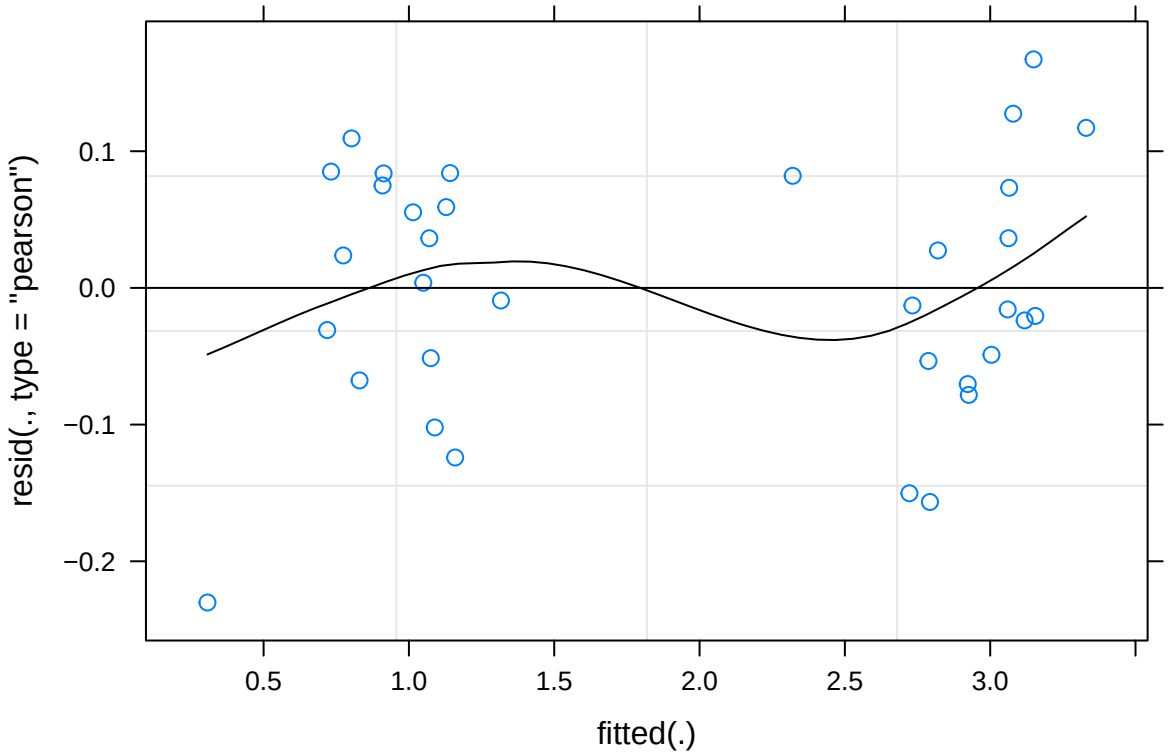
Sex differences analysis exclusively for animals that were littermates

```
litter <- subset(sex_diff,  animal %in% c("1M","2M","3M","4M","5M","6M","7M","8M","9M","18F","19F","20F
litter
```

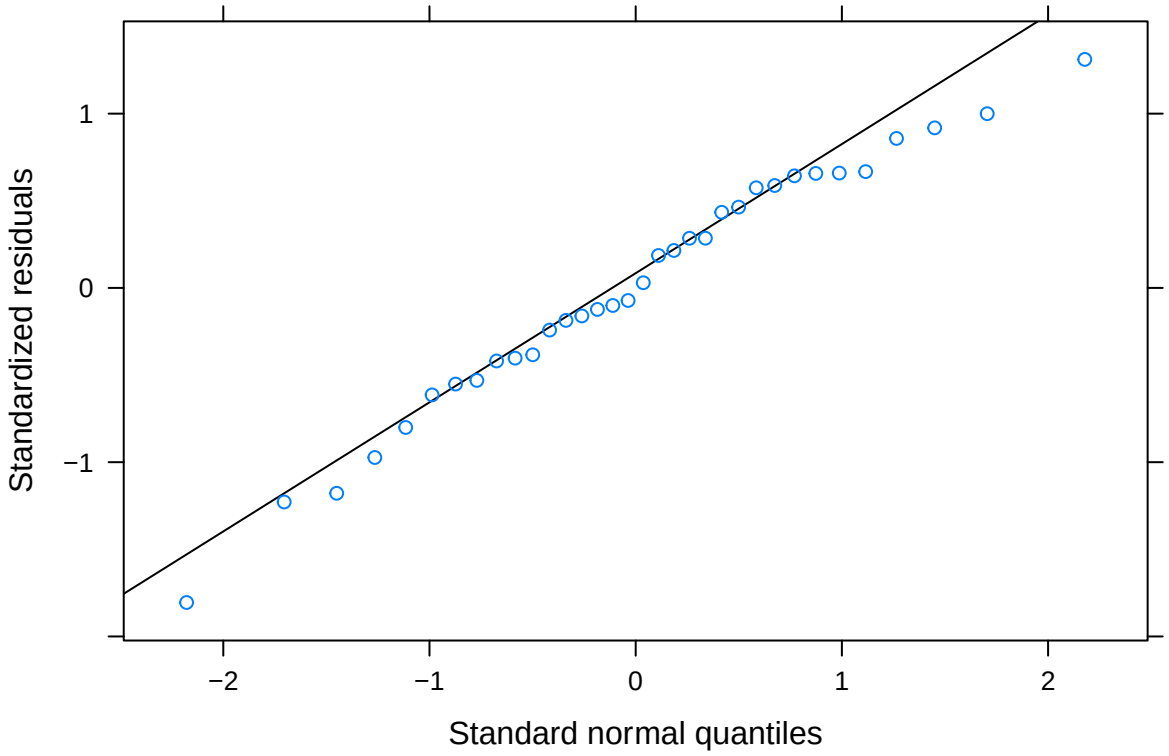
```
##    animal raw_conc tissue_weight body_wt  dose     stage group tissue   conc_wt
## 1      1M  56.5895          20.1    27.2 20.60      male  male  brain  2.815398
## 2      2M  22.0798          10.3    30.1 18.60      male  male  brain  2.143670
## 3      3M  36.7582          13.7    28.7 19.50      male  male  brain  2.683080
## 4      4M  49.5114          15.1    25.8 21.70      male  male  brain  3.278901
## 5      5M  87.4161          31.4    26.1 20.00      male  male  brain  2.783952
## 6      6M  36.5960          14.7    27.3 19.10      male  male  brain  2.489524
## 7      7M  41.6730          14.3    26.2 19.90      male  male  brain  2.914196
## 8      8M  62.8960          20.8    23.7 22.00      male  male  brain  3.023846
## 9      9M  35.5456          15.7    27.2 19.20      male  male  brain  2.264051
## 22    18F 116.9270          43.7    21.3 20.40    estrus highE  brain  2.675675
## 23    19F  15.7652          14.6    22.9 19.00    estrus highE  brain  1.079808
## 24    20F  40.6623          11.0    20.9 20.80 proestrus highE  brain  3.696573
## 25    21F  43.5806          15.2    20.4 21.30 proestrus highE  brain  2.867145
## 26    22F  64.0701          32.2    23.2 18.70    estrus highE  brain  1.989755
## 27    24F  57.2307          16.8    21.2 20.50 proestrus highE  brain  3.406589
## 28    25F  25.7378           9.5    20.3 21.40    estrus highE  brain  2.709242
## 29    26F  23.9826          10.8    21.7 20.00    estrus highE  brain  2.220611
## 38     1M 104.7333           3.8    27.2 20.55      male  male retina 27.561395
## 39     2M  86.2347           5.0    30.1 18.57      male  male retina 17.246940
## 40     3M  98.7759           4.0    28.7 19.48      male  male retina 24.693975
## 41     4M  88.3202           4.0    25.8 21.67      male  male retina 22.080050
## 42     5M  99.2028           4.3    26.1 20.00      male  male retina 23.070419
```

```
## 43     6M  97.7141          7.0    27.3 19.12      male  male retina 13.959157
## 44     7M  99.8877          5.2    26.2 19.92      male  male retina 19.209173
## 45     8M 119.6988          5.7    23.7 22.03      male  male retina 20.999789
## 46     9M  74.6145          5.7    27.2 19.19      male  male retina 13.090263
## 59    18F  84.9179          4.9    21.3 20.38    estrus highE retina 17.330184
## 60    19F  51.9343          4.7    22.9 18.95    estrus highE retina 11.049851
## 61    20F 119.3678          3.8    20.9 20.77 proestrus highE retina 31.412579
## 62    21F 117.5767          5.3    20.4 21.27 proestrus highE retina 22.184283
## 63    22F  77.3790          5.1    23.2 18.71    estrus highE retina 15.172353
## 64    24F 114.9038          5.0    21.2 20.47 proestrus highE retina 22.980760
## 65    25F  93.1777          5.4    20.3 21.38    estrus highE retina 17.255130
## 66    26F  61.5810          4.0    21.7 20.00    estrus highE retina 15.395250
##    log_conc_wt log_conc_raw
## 1   1.03510364     4.035823
## 2   0.76251927     3.094663
## 3   0.98696550     3.604361
## 4   1.18750820     3.902203
## 5   1.02387158     4.470679
## 6   0.91209145     3.599939
## 7   1.06959390     3.729853
## 8   1.10652958     4.141483
## 9   0.81715567     3.570816
## 22  0.98420171     4.761550
## 23  0.07678345     2.757805
## 24  1.30740610     3.705301
## 25  1.05331667     3.774612
## 26  0.68801134     4.159978
## 27  1.22571158     4.047090
## 28  0.99666893     3.247961
## 29  0.79778243     3.177329
## 38  3.31641605     4.651417
## 39  2.84763474     4.457073
## 40  3.20655929     4.592854
## 41  3.09467449     4.480969
## 42  3.13855122     4.597166
## 43  2.63613572     4.582046
## 44  2.95538793     4.604047
## 45  3.04451241     4.784979
## 46  2.57186868     4.312335
## 59  2.85244970     4.441685
## 60  2.40241695     3.949979
## 61  3.44720842     4.782209
## 62  3.09938407     4.767091
## 63  2.71947489     4.348715
## 64  3.13465734     4.744095
## 65  2.84810947     4.534508
## 66  2.73405902     4.120353
```

```
lmer_litt <- lmer(log(conc_wt) ~ group*tissue+(1|animal), data =litter)
plot(lmer_litt, type=c("p","smooth"), col.line=1)
```




```
lattice::qqmath(lmer_litt)
```




```
anova(lmer_litt)
```

```
## Type III Analysis of Variance Table with Satterthwaite's method
##              Sum Sq Mean Sq NumDF DenDF   F value Pr(>F)
## group         0.007   0.007     1    15    0.4345 0.5198
## tissue       33.942  33.942     1    15 2087.8664 <2e-16 ***
## group:tissue  0.001   0.001     1    15    0.0716 0.7927
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
emmeans::emmeans(lmer_litt, pairwise ~ tissue| group)
```

```
## $emmeans
## group = male:
##  tissue emmean     SE   df lower.CL upper.CL
##  retina  2.979 0.0945 18.3    2.781     3.18
##  brain   0.989 0.0945 18.3    0.791     1.19
##
## group = highE:
##  tissue emmean     SE   df lower.CL upper.CL
##  retina  2.905 0.1002 18.3    2.694     3.12
##  brain   0.891 0.1002 18.3    0.681     1.10
##
## Degrees-of-freedom method: kenward-roger
## Results are given on the log (not the response) scale.
```

```
## Confidence level used: 0.95
##
## $contrasts
## group = male:
##  contrast       estimate     SE df t.ratio p.value
##  retina - brain     1.99 0.0601 15  33.109  <.0001
##
## group = highE:
##  contrast       estimate     SE df t.ratio p.value
##  retina - brain     2.01 0.0638 15  31.583  <.0001
##
## Degrees-of-freedom method: kenward-roger
## Results are given on the log (not the response) scale.
```

```
emmeans::emmeans(lmer_litt, pairwise ~ group| tissue)
```

```
## $emmeans
## tissue = retina:
##  group emmean     SE   df lower.CL upper.CL
##  male   2.979 0.0945 18.3    2.781     3.18
##  highE  2.905 0.1002 18.3    2.694     3.12
##
## tissue = brain:
##  group emmean     SE   df lower.CL upper.CL
##  male   0.989 0.0945 18.3    0.791     1.19
##  highE  0.891 0.1002 18.3    0.681     1.10
##
## Degrees-of-freedom method: kenward-roger
## Results are given on the log (not the response) scale.
## Confidence level used: 0.95
##
## $contrasts
## tissue = retina:
##  contrast     estimate    SE   df t.ratio p.value
##  male - highE   0.0744 0.138 18.3   0.540  0.5958
##
## tissue = brain:
##  contrast     estimate    SE   df t.ratio p.value
##  male - highE   0.0978 0.138 18.3   0.710  0.4866
##
## Degrees-of-freedom method: kenward-roger
## Results are given on the log (not the response) scale.
```

```
shapiro.test(resid(lmer_litt))
```

```
##
##  Shapiro-Wilk normality test
##
## data:  resid(lmer_litt)
## W = 0.97782, p-value = 0.7028
```

Visualizations

```
SumStat_sexlitt <- dplyr::summarise(group_by(litter, tissue, group),
n = n(),
mean_conc_wt = mean(conc_wt),
sd_conc_wt = sd(conc_wt),
se_conc_wt = sd_conc_wt/sqrt(n),
log_conc = mean(log(conc_wt)),
sd_conc_log = sd(log(conc_wt)),
se_conc_log = sd_conc_log/sqrt(n),
mean_wt = mean(body_wt),
sd_wt = sd(body_wt),
mean_dose = mean(dose),
sd_dose = sd(dose),
)
```

```
## `summarise()` has grouped output by 'tissue'. You can override using the
## `.groups` argument.
```

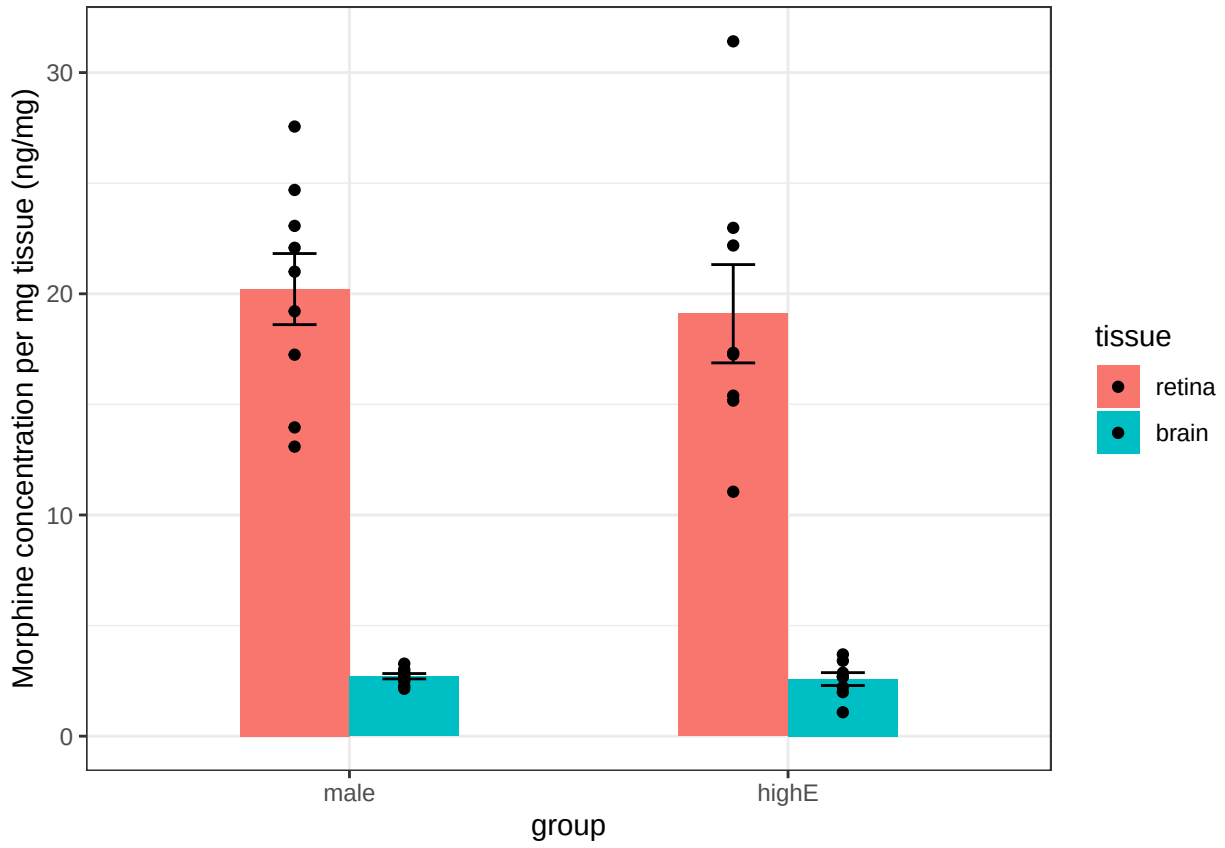
```
SumStat_sexlitt
```

```
## # A tibble: 4 x 13
## # Groups:   tissue [2]
##   tissue group     n mean_conc~1 sd_co~2 se_co~3 log_c~4 sd_co~5 se_co~6 mean_wt
##   <fct>  <fct> <int>       <dbl>   <dbl>   <dbl>   <dbl>   <dbl>   <dbl>   <dbl>
## 1 retina male      9       20.2    4.82    1.61    2.98    0.252  0.0842    26.9
## 2 retina highE     8       19.1    6.29    2.22    2.90    0.318  0.112     21.5
## 3 brain  male      9        2.71   0.362   0.121   0.989   0.137  0.0456    26.9
## 4 brain  highE     8        2.58   0.825   0.292   0.891   0.386  0.137     21.5
## # ... with 3 more variables: sd_wt <dbl>, mean_dose <dbl>, sd_dose <dbl>, and
## #   abbreviated variable names 1: mean_conc_wt, 2: sd_conc_wt, 3: se_conc_wt,
## #   4: log_conc, 5: sd_conc_log, 6: se_conc_log
```

```
plot_tissue_litt <- ggplot() +
  geom_bar(data=SumStat_sexlitt, aes(x=group, y=mean_conc_wt, fill=tissue), stat="identity", position=po
  geom_errorbar(data=SumStat_sexlitt, aes(x=group,ymin=mean_conc_wt - se_conc_wt, ymax=mean_conc_wt + se
  geom_point(data=litter, aes(x=group, y=conc_wt, fill=tissue), position=position_dodge(width=0.5)) +
  scale_y_continuous(name="Morphine concentration per mg tissue (ng/mg)") + theme_bw()
```

```
## Warning in geom_errorbar(data = SumStat_sexlitt, aes(x = group, ymin =
## mean_conc_wt - : Ignoring unknown aesthetics: fill
```

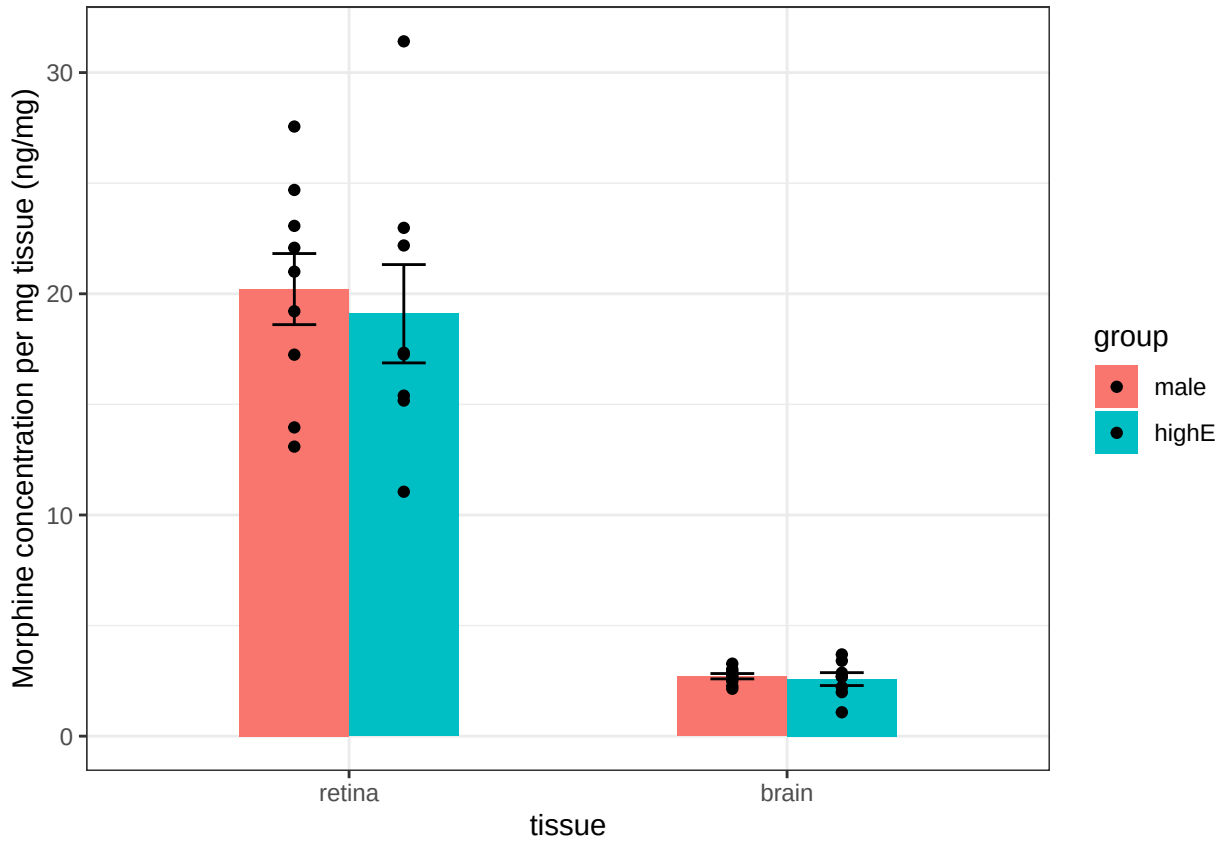
```
plot_tissue_litt
```




```
plot_stage_litt <- ggplot() +
  geom_bar(data=SumStat_sexlitt, aes(x=tissue, y=mean_conc_wt, fill=group), stat="identity", position=po
  geom_errorbar(data=SumStat_sexlitt, aes(x=tissue,ymin=mean_conc_wt - se_conc_wt, ymax=mean_conc_wt + s
  geom_point(data=litter, aes(x=tissue, y=conc_wt, fill=group), position=position_dodge(width=0.5)) +
  scale_y_continuous(name="Morphine concentration per mg tissue (ng/mg)") + theme_bw()
```

```
## Warning in geom_errorbar(data = SumStat_sexlitt, aes(x = tissue, ymin =
## mean_conc_wt - : Ignoring unknown aesthetics: fill
```

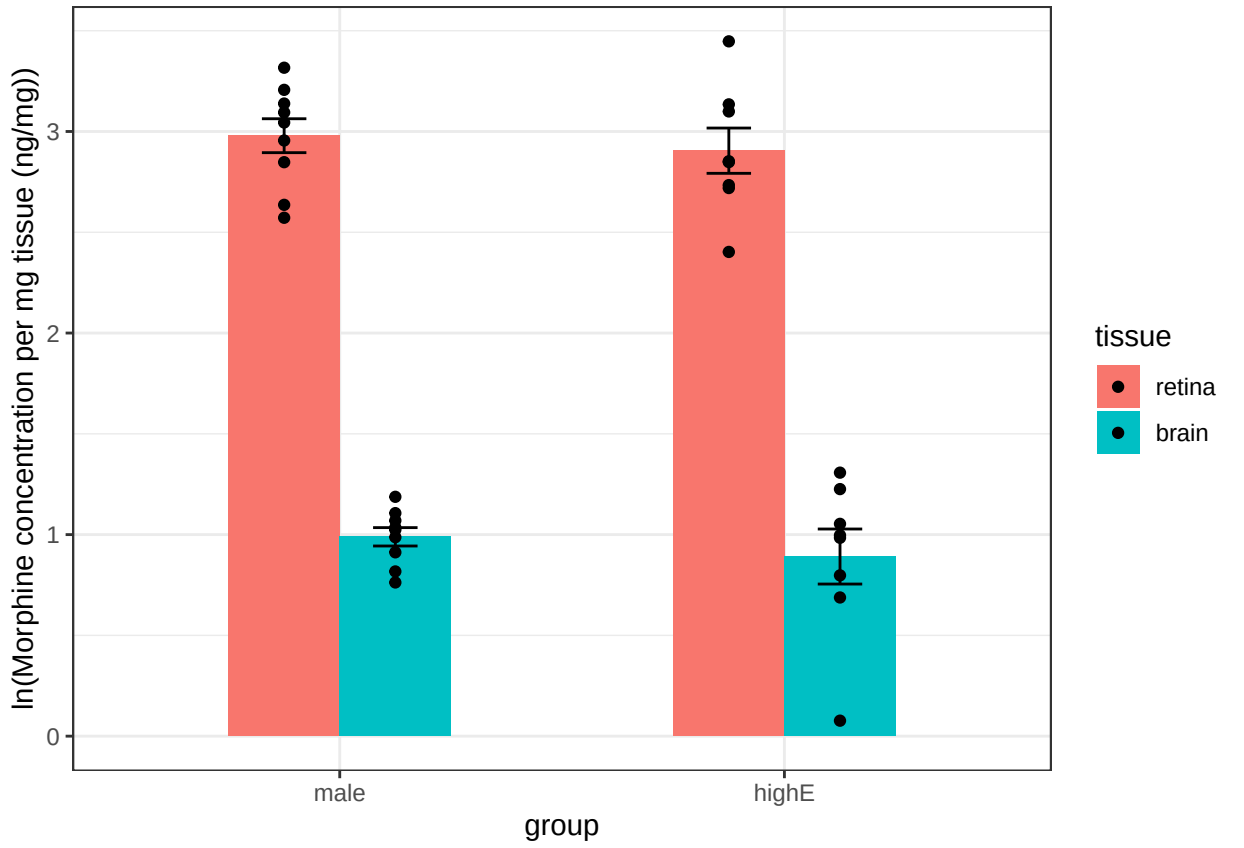
```
plot_stage_litt
```




```
plot_tissueL_litt <- ggplot() +
  geom_bar(data=SumStat_sexlitt, aes(x=group, y=log_conc, fill=tissue), stat="identity", position=posit
  geom_errorbar(data=SumStat_sexlitt, aes(x=group,ymin=log_conc - se_conc_log, ymax=log_conc + se_conc_
  scale_y_continuous(name="ln(Morphine concentration per mg tissue (ng/mg))") + theme_bw()
```

```
## Warning in geom_errorbar(data = SumStat_sexlitt, aes(x = group, ymin = log_conc
## - : Ignoring unknown aesthetics: fill
```

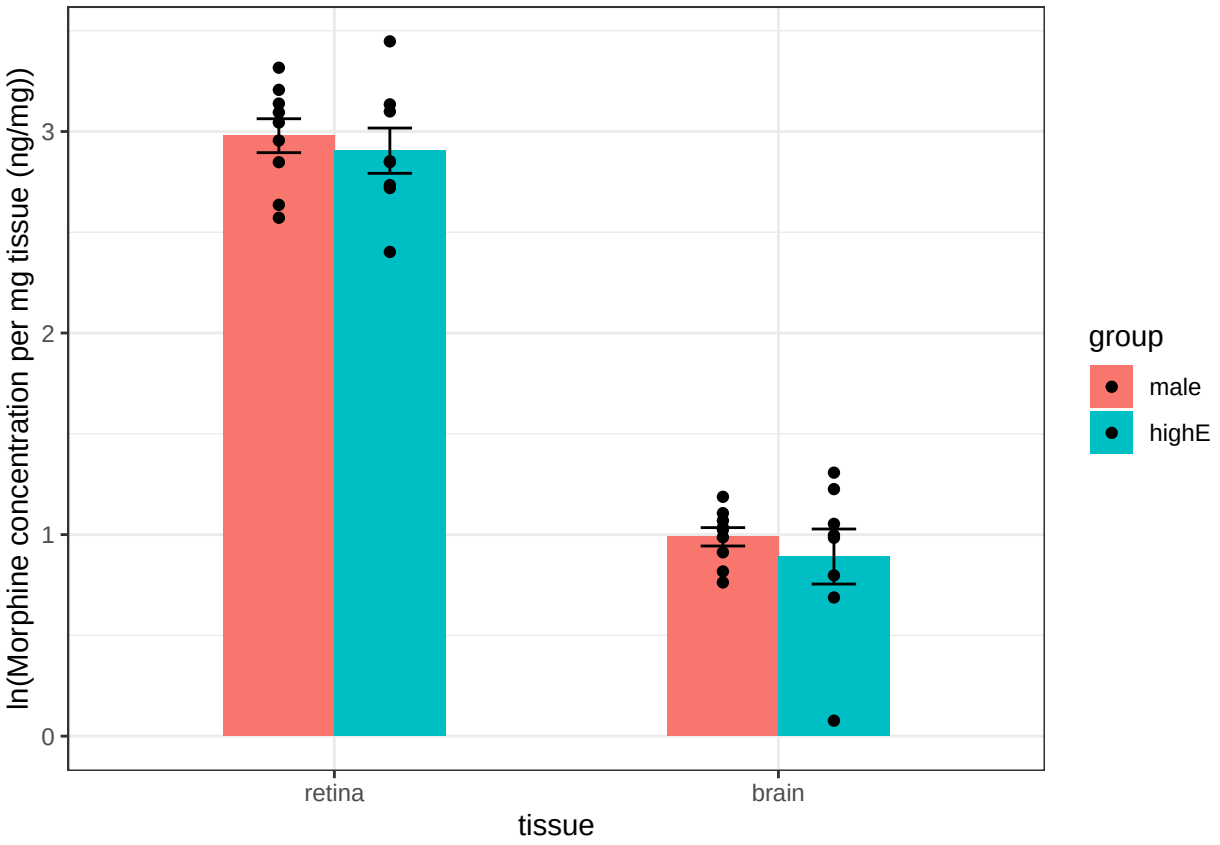
```
plot_tissueL_litt
```




```
plot_stageL_litt <- ggplot() +
  geom_bar(data=SumStat_sexlitt, aes(x=tissue, y=log_conc, fill=group), stat="identity", position=posit
  geom_errorbar(data=SumStat_sexlitt, aes(x=tissue,ymin=log_conc - se_conc_log, ymax=log_conc + se_conc
  scale_y_continuous(name="ln(Morphine concentration per mg tissue (ng/mg))") + theme_bw()
```

```
## Warning in geom_errorbar(data = SumStat_sexlitt, aes(x = tissue, ymin = log_conc
## - : Ignoring unknown aesthetics: fill
```

```
plot_stageL_litt
```



Comparisons between estrus stages

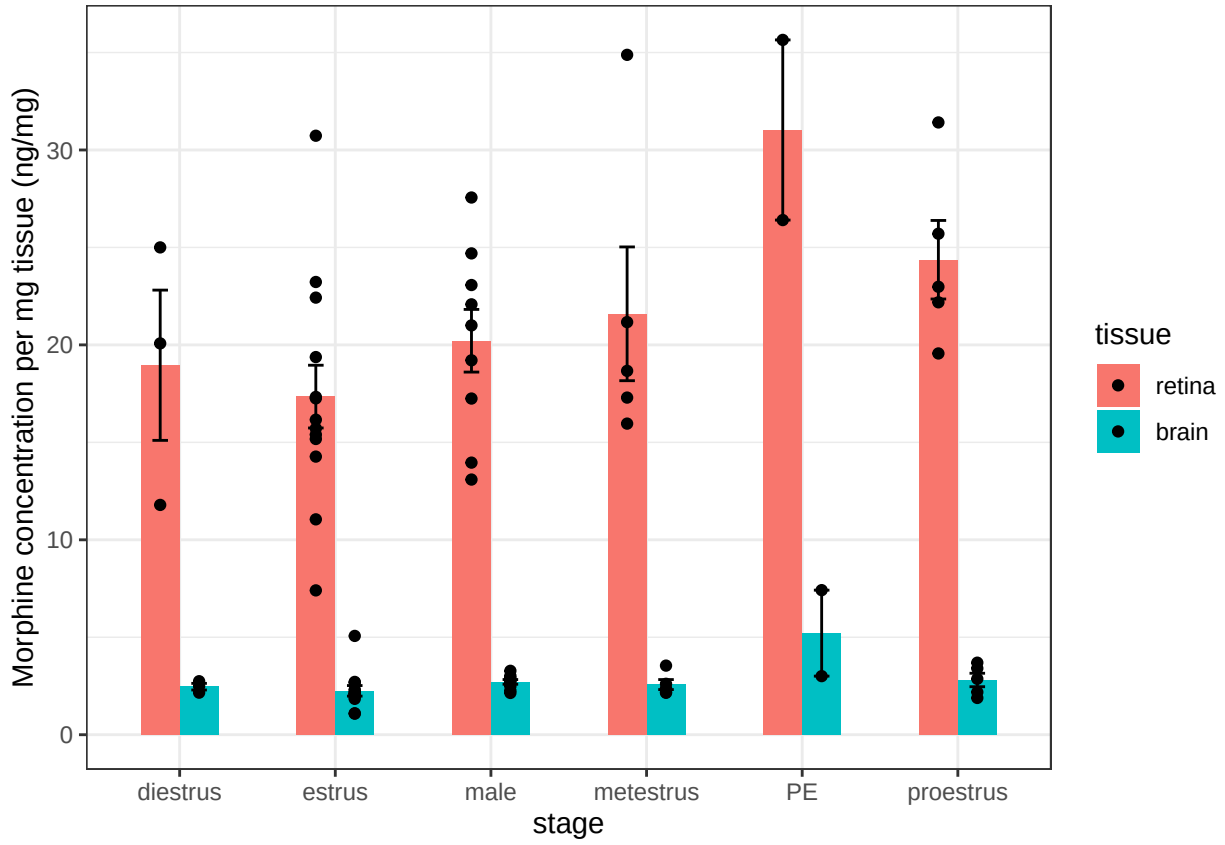
```
SumStat_sexstage <- dplyr::summarise(group_by(sex_diff, tissue, stage),
n = n(),
mean_conc_wt = mean(conc_wt),
sd_conc_wt = sd(conc_wt),
se_conc_wt = sd_conc_wt/sqrt(n),
log_conc = mean(log(conc_wt)),
sd_conc_log = sd(log(conc_wt)),
se_conc_log = sd_conc_log/sqrt(n),
mean_wt = mean(body_wt),
sd_wt = sd(body_wt),
mean_dose = mean(dose),
sd_dose = sd(dose),
)
```

```
## `summarise()` has grouped output by 'tissue'. You can override using the
## `.groups` argument.
```

```
plot_stage_tissue <- ggplot() +
  geom_bar(data=SumStat_sexstage, aes(x=stage, y=mean_conc_wt, fill=tissue), stat="identity", position=
  geom_errorbar(data=SumStat_sexstage, aes(x=stage,ymin=mean_conc_wt - se_conc_wt, ymax=mean_conc_wt + 
  geom_point(data=sex_diff, aes(x=stage, y=conc_wt, fill=tissue), position=position_dodge(width=0.5)) +
  scale_y_continuous(name="Morphine concentration per mg tissue (ng/mg)") + theme_bw()
```

```
## Warning in geom_errorbar(data = SumStat_sexstage, aes(x = stage, ymin =
## mean_conc_wt - : Ignoring unknown aesthetics: fill
```

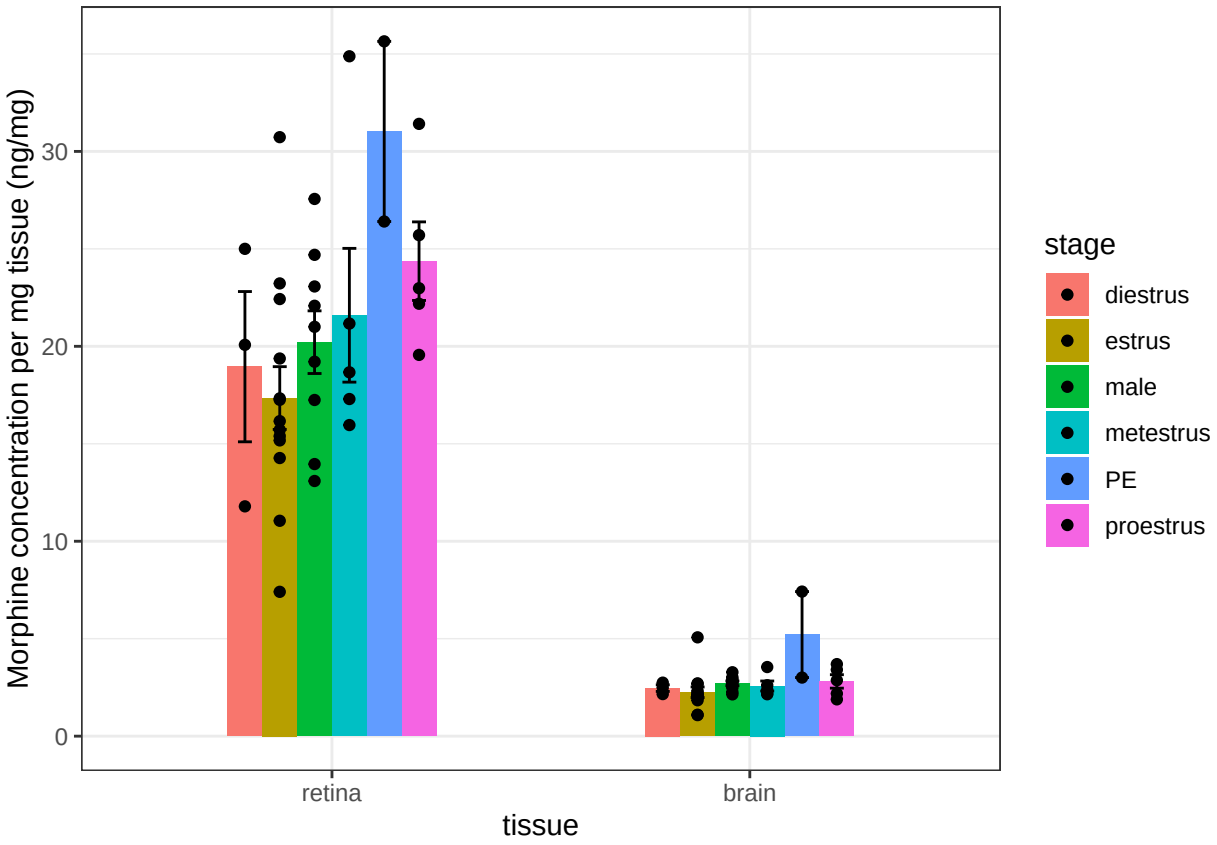
```
plot_stage_tissue
```



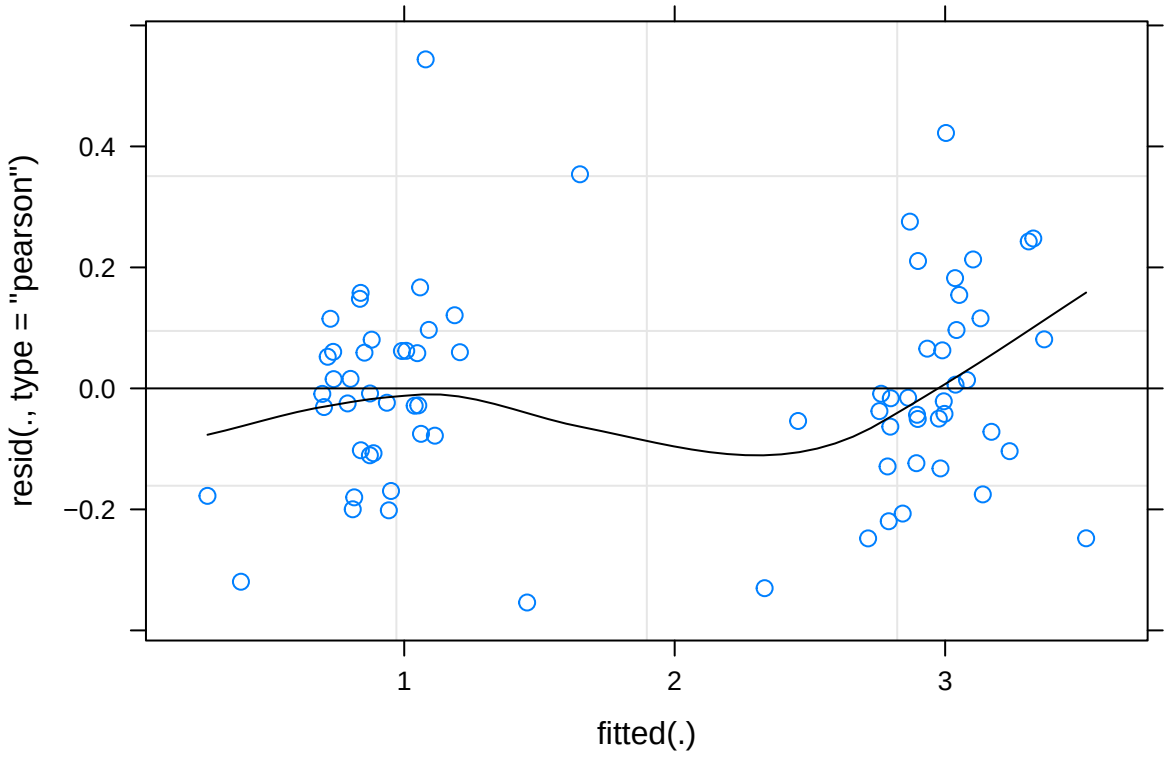

```
plot_stages <- ggplot() +
  geom_bar(data=SumStat_sexstage, aes(x=tissue, y=mean_conc_wt, fill=stage), stat="identity", position=
  geom_errorbar(data=SumStat_sexstage, aes(x=tissue,ymin=mean_conc_wt - se_conc_wt, ymax=mean_conc_wt +
  geom_point(data=sex_diff, aes(x=tissue, y=conc_wt, fill=stage), position=position_dodge(width=0.5)) +
  scale_y_continuous(name="Morphine concentration per mg tissue (ng/mg)") + theme_bw()
```

```
## Warning in geom_errorbar(data = SumStat_sexstage, aes(x = tissue, ymin =
## mean_conc_wt - : Ignoring unknown aesthetics: fill
```

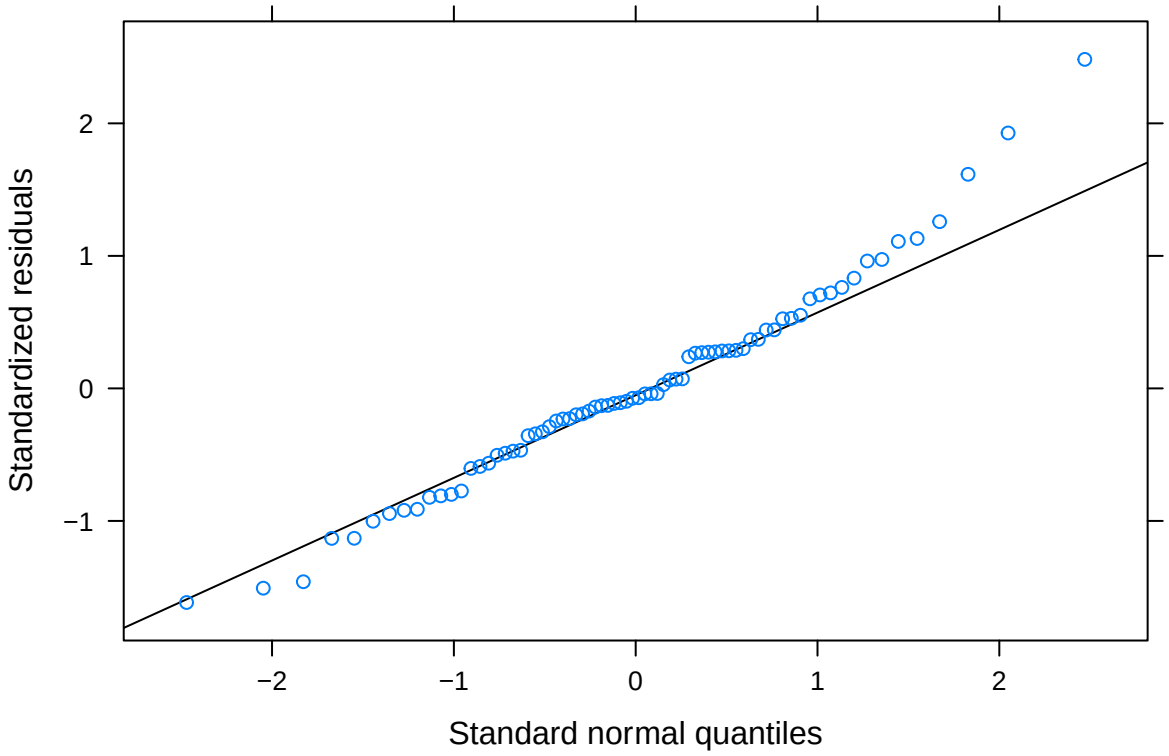
```
plot_stages
```




```
lmer_stage <- lmer(log(conc_wt) ~ stage*tissue+(1|animal), data =sex_diff)
plot(lmer_stage, type=c("p","smooth"), col.line=1)
```




```
lattice::qqmath(lmer_stage)
```




```
anova(lmer_stage)
```

```
## Type III Analysis of Variance Table with Satterthwaite's method
##              Sum Sq Mean Sq NumDF DenDF   F value  Pr(>F)
## stage         0.789   0.158     5    31    3.2863 0.01701 *
## tissue       52.376  52.376     1    31 1091.4373 < 2e-16 ***
## stage:tissue  0.102   0.020     5    31    0.4244 0.82809
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
emmeans::emmeans(lmer_stage, pairwise ~ tissue| stage)
```

```
## $emmeans
## stage = diestrus:
##  tissue emmean     SE   df lower.CL upper.CL
##  retina  2.895 0.1753 50.4    2.543    3.247
##  brain   0.897 0.1753 50.4    0.545    1.249
##
## stage = estrus:
##  tissue emmean     SE   df lower.CL upper.CL
##  retina  2.800 0.0842 50.4    2.631    2.969
##  brain   0.740 0.0842 50.4    0.571    0.909
##
## stage = male:
```

```
##  tissue emmean     SE   df lower.CL upper.CL
##  retina  2.979 0.1012 50.4    2.776    3.182
##  brain   0.989 0.1012 50.4    0.786    1.192
##
## stage = metestrus:
##  tissue emmean     SE   df lower.CL upper.CL
##  retina  3.030 0.1358 50.4    2.758    3.303
##  brain   0.927 0.1358 50.4    0.655    1.200
##
## stage = PE:
##  tissue emmean     SE   df lower.CL upper.CL
##  retina  3.424 0.2147 50.4    2.993    3.855
##  brain   1.552 0.2147 50.4    1.121    1.983
##
## stage = proestrus:
##  tissue emmean     SE   df lower.CL upper.CL
##  retina  3.180 0.1358 50.4    2.908    3.453
##  brain   1.001 0.1358 50.4    0.728    1.273
##
## Degrees-of-freedom method: kenward-roger
## Results are given on the log (not the response) scale.
## Confidence level used: 0.95
##
## $contrasts
## stage = diestrus:
##  contrast       estimate     SE df t.ratio p.value
##  retina - brain     2.00 0.1789 31  11.171  <.0001
##
## stage = estrus:
##  contrast       estimate     SE df t.ratio p.value
##  retina - brain     2.06 0.0859 31  23.974  <.0001
##
## stage = male:
##  contrast       estimate     SE df t.ratio p.value
##  retina - brain     1.99 0.1033 31  19.271  <.0001
##
## stage = metestrus:
##  contrast       estimate     SE df t.ratio p.value
##  retina - brain     2.10 0.1385 31  15.179  <.0001
##
## stage = PE:
##  contrast       estimate     SE df t.ratio p.value
##  retina - brain     1.87 0.2191 31   8.543  <.0001
##
## stage = proestrus:
##  contrast       estimate     SE df t.ratio p.value
##  retina - brain     2.18 0.1385 31  15.732  <.0001
##
## Degrees-of-freedom method: kenward-roger
## Results are given on the log (not the response) scale.
```

```
emmeans::emmeans(lmer_stage, pairwise ~ stage| tissue)
```

```
## $emmeans
```

```
## tissue = retina:
##  stage     emmean     SE   df lower.CL upper.CL
##  diestrus   2.895 0.1753 50.4    2.543    3.247
##  estrus     2.800 0.0842 50.4    2.631    2.969
##  male       2.979 0.1012 50.4    2.776    3.182
##  metestrus  3.030 0.1358 50.4    2.758    3.303
##  PE         3.424 0.2147 50.4    2.993    3.855
##  proestrus  3.180 0.1358 50.4    2.908    3.453
##
## tissue = brain:
##  stage     emmean     SE   df lower.CL upper.CL
##  diestrus   0.897 0.1753 50.4    0.545    1.249
##  estrus     0.740 0.0842 50.4    0.571    0.909
##  male       0.989 0.1012 50.4    0.786    1.192
##  metestrus  0.927 0.1358 50.4    0.655    1.200
##  PE         1.552 0.2147 50.4    1.121    1.983
##  proestrus  1.001 0.1358 50.4    0.728    1.273
##
## Degrees-of-freedom method: kenward-roger
## Results are given on the log (not the response) scale.
## Confidence level used: 0.95
##
## $contrasts
## tissue = retina:
##  contrast              estimate    SE   df t.ratio p.value
##  diestrus - estrus       0.0953 0.194 50.4   0.490  0.9963
##  diestrus - male        -0.0839 0.202 50.4  -0.414  0.9983
##  diestrus - metestrus   -0.1351 0.222 50.4  -0.609  0.9899
##  diestrus - PE          -0.5283 0.277 50.4  -1.907  0.4102
##  diestrus - proestrus   -0.2850 0.222 50.4  -1.286  0.7913
##  estrus - male          -0.1792 0.132 50.4  -1.361  0.7494
##  estrus - metestrus     -0.2305 0.160 50.4  -1.443  0.7012
##  estrus - PE            -0.6237 0.231 50.4  -2.705  0.0920
##  estrus - proestrus     -0.3804 0.160 50.4  -2.381  0.1825
##  male - metestrus       -0.0513 0.169 50.4  -0.303  0.9996
##  male - PE              -0.4445 0.237 50.4  -1.873  0.4302
##  male - proestrus       -0.2012 0.169 50.4  -1.188  0.8404
##  metestrus - PE         -0.3932 0.254 50.4  -1.548  0.6354
##  metestrus - proestrus  -0.1499 0.192 50.4  -0.781  0.9695
##  PE - proestrus          0.2433 0.254 50.4   0.958  0.9289
##
## tissue = brain:
##  contrast              estimate    SE   df t.ratio p.value
##  diestrus - estrus       0.1572 0.194 50.4   0.809  0.9646
##  diestrus - male        -0.0918 0.202 50.4  -0.454  0.9974
##  diestrus - metestrus   -0.0301 0.222 50.4  -0.136  1.0000
##  diestrus - PE          -0.6549 0.277 50.4  -2.363  0.1889
##  diestrus - proestrus   -0.1034 0.222 50.4  -0.466  0.9971
##  estrus - male          -0.2491 0.132 50.4  -1.892  0.4188
##  estrus - metestrus     -0.1873 0.160 50.4  -1.172  0.8477
##  estrus - PE            -0.8122 0.231 50.4  -3.522  0.0112
##  estrus - proestrus     -0.2606 0.160 50.4  -1.632  0.5821
##  male - metestrus        0.0618 0.169 50.4   0.365  0.9991
##  male - PE              -0.5631 0.237 50.4  -2.373  0.1854
```

```
##  male - proestrus       -0.0116 0.169 50.4  -0.068  1.0000
##  metestrus - PE         -0.6249 0.254 50.4  -2.460  0.1557
##  metestrus - proestrus  -0.0733 0.192 50.4  -0.382  0.9989
##  PE - proestrus          0.5515 0.254 50.4   2.172  0.2690
##
## Degrees-of-freedom method: kenward-roger
## Results are given on the log (not the response) scale.
## P value adjustment: tukey method for comparing a family of 6 estimates
```

```
shapiro.test(resid(lmer_stage))
```

```
##
##  Shapiro-Wilk normality test
##
## data:  resid(lmer_stage)
## W = 0.97701, p-value = 0.196
```