sex_differences

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Visualizations for potential sex and tissue-dependent differences

```
sex_diff <- read.csv("../data/morphine_retina_cx_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)
str(sex_diff)
```

```
## 'data.frame':    74 obs. of  10 variables:
##  $ animal       : chr  "1M" "2M" "3M" "4M" ...
##  $ 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        : chr  "male" "male" "male" "male" ...
##  $ tissue       : chr  "brain" "brain" "brain" "brain" ...
##  $ 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 ...
```

```
sex_diff
```

```
##    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
## 10     1F  43.5887          20.5    22.0 19.10    estrus highE  brain  2.126278
## 11     3F  32.3785          15.0    19.9 21.10  diestrus  lowE  brain  2.158567
## 12     5F  19.3532          17.6    19.9 21.30    estrus highE  brain  1.099614
## 13     6F  26.3844          10.1    21.4 19.80 metestrus  lowE  brain  2.612317
## 14     7F  35.2902          15.2    23.2 18.30    estrus highE  brain  2.321724
## 15     8F  88.8275          41.3    20.2 21.00 metestrus  lowE  brain  2.150787
## 16    11F  20.2764           4.0    20.6 19.80    estrus highE  brain  5.069100
## 17    12F  28.8012          14.7    22.3 19.10    estrus highE  brain  1.959265
## 18    13F  26.7006           3.6    19.3 22.10        PE highE  brain  7.416833
```

```
## 19    14F  36.0702         19.6    22.1 19.30    estrus highE  brain  1.840316
## 20    15F  39.9838         18.3    21.1 20.20 proestrus highE  brain  2.184907
## 21    16F  20.5701         10.9    21.6 19.70 proestrus highE  brain  1.887165
## 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
## 30    27F  42.9962         18.1    21.1 19.30 metestrus  lowE  brain  2.375481
## 31    28F  32.9760          9.3    19.6 20.80 metestrus  lowE  brain  3.545806
## 32    29F  22.0195         10.1    21.4 19.10 metestrus  lowE  brain  2.180149
## 33    30F  24.2505         11.6    19.5 20.90    estrus highE  brain  2.090560
## 34    31F  63.1233         21.0    22.5 19.50        PE highE  brain  3.005871
## 35    32F  70.8384         25.8    21.2 20.70  diestrus  lowE  brain  2.745674
## 36    33F  55.0222         22.1    21.8 20.10  diestrus  lowE  brain  2.489692
## 37    34F  53.5313         25.5    22.2 19.80    estrus highE  brain  2.099267
## 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
## 47     1F  84.0422          5.2    22.0 19.09    estrus highE retina 16.161962
## 48     3F  56.5895          4.8    19.9 21.10  diestrus  lowE retina 11.789479
## 49     5F  39.9900          5.4    19.9 21.31    estrus highE retina  7.405556
## 50     6F  89.9399          5.2    21.4 19.81 metestrus  lowE retina 17.296135
## 51     7F  67.0640          4.7    23.2 18.28    estrus highE retina 14.268936
## 52     8F  95.7628          6.0    20.2 20.99 metestrus  lowE retina 15.960467
## 53    11F  94.9356          4.9    20.6 19.81    estrus highE retina 19.374612
## 54    12F  69.1372          4.4    22.3 19.10    estrus highE retina 15.713000
## 55    13F 110.8945          4.2    19.3 22.07        PE highE retina 26.403452
## 56    14F  95.2281          4.1    22.1 19.28    estrus highE retina 23.226366
## 57    15F 120.8068          4.7    21.1 20.19 proestrus highE retina 25.703574
## 58    16F  97.8027          5.0    21.6 19.72 proestrus highE retina 19.560540
## 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
## 67    27F  80.2655          4.3    21.1 19.34 metestrus  lowE retina 18.666395
## 68    28F 125.5700          3.6    19.6 20.82 metestrus  lowE retina 34.880556
## 69    29F 112.1923          5.3    21.4 19.07 metestrus  lowE retina 21.168358
## 70    30F 107.6408          4.8    19.5 20.92    estrus highE retina 22.425167
## 71    31F 146.1486          4.1    22.5 19.51        PE highE retina 35.646000
## 72    32F 100.0052          4.0    21.2 20.71  diestrus  lowE retina 25.001300
```

```
## 73    33F 104.3988          5.2    21.8 20.14  diestrus  lowE retina 20.076692
## 74    34F 107.5554          3.5    22.2 19.77    estrus highE retina 30.730114
##    log_conc_wt
## 1   1.03510364
## 2   0.76251927
## 3   0.98696550
## 4   1.18750820
## 5   1.02387158
## 6   0.91209145
## 7   1.06959390
## 8   1.10652958
## 9   0.81715567
## 10  0.75437306
## 11  0.76944442
## 12  0.09495888
## 13  0.96023750
## 14  0.84230988
## 15  0.76583379
## 16  1.62316329
## 17  0.67256956
## 18  2.00375219
## 19  0.60993747
## 20  0.78157331
## 21  0.63507578
## 22  0.98420171
## 23  0.07678345
## 24  1.30740610
## 25  1.05331667
## 26  0.68801134
## 27  1.22571158
## 28  0.99666893
## 29  0.79778243
## 30  0.86519980
## 31  1.26576562
## 32  0.77939300
## 33  0.73743214
## 34  1.10056752
## 35  1.01002673
## 36  0.91215913
## 37  0.74158808
## 38  3.31641605
## 39  2.84763474
## 40  3.20655929
## 41  3.09467449
## 42  3.13855122
## 43  2.63613572
## 44  2.95538793
## 45  3.04451241
## 46  2.57186868
## 47  2.78266043
## 48  2.46720754
## 49  2.00223047
## 50  2.85048304
## 51  2.65808488
```

```
## 52  2.77011483
## 53  2.96396356
## 54  2.75448840
## 55  3.27349477
## 56  3.14528809
## 57  3.24663007
## 58  2.97351427
## 59  2.85244970
## 60  2.40241695
## 61  3.44720842
## 62  3.09938407
## 63  2.71947489
## 64  3.13465734
## 65  2.84810947
## 66  2.73405902
## 67  2.92672487
## 68  3.55192953
## 69  3.05250754
## 70  3.11018384
## 71  3.57363694
## 72  3.21892782
## 73  2.99955956
## 74  3.42524310
```

```
#test for outliers
test_out <- rosnerTest(sex_diff$conc_wt,
  k = 4
)
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] 0
##
## $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_sexdiff <- dplyr::summarise(group_by(sex_diff, 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_sexdiff
```

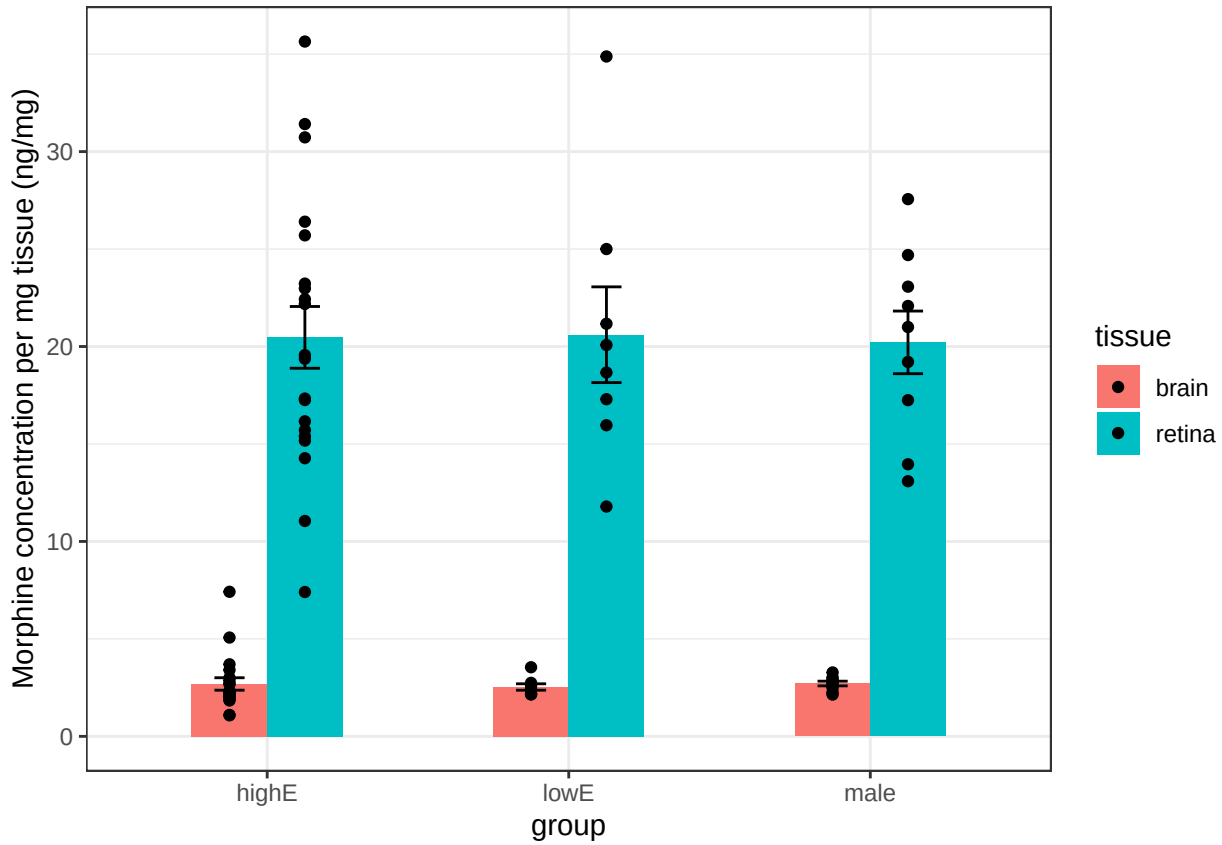
```
## # A tibble: 6 x 13
```

```
## # Groups:   tissue [2]
##   tissue group     n mean_conc_wt sd_conc_wt se_conc_wt log_conc sd_conc_log
##   <chr>  <chr> <int>        <dbl>      <dbl>      <dbl>    <dbl>       <dbl>
## 1 brain  highE    20         2.69       1.43      0.320    0.886       0.445
## 2 brain  lowE      8         2.53      0.465      0.164    0.916       0.168
## 3 brain  male      9         2.71      0.362      0.121    0.989       0.137
## 4 retina highE    20         20.5       7.08       1.58     2.96       0.373
## 5 retina lowE      8         20.6       6.95       2.46     2.98       0.320
## 6 retina male      9         20.2       4.82       1.61     2.98       0.252
## # ... with 5 more variables: se_conc_log <dbl>, mean_wt <dbl>, sd_wt <dbl>,
## #   mean_dose <dbl>, sd_dose <dbl>
```

```
plot_tissue <- ggplot() +
  geom_bar(data=SumStat_sexdiff, aes(x=group, y=mean_conc_wt, fill=tissue), stat="identity", position=po
  geom_errorbar(data=SumStat_sexdiff, aes(x=group,ymin=mean_conc_wt - se_conc_wt, ymax=mean_conc_wt + s
  geom_point(data=sex_diff, 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: Ignoring unknown aesthetics: fill
```

```
plot_tissue
```

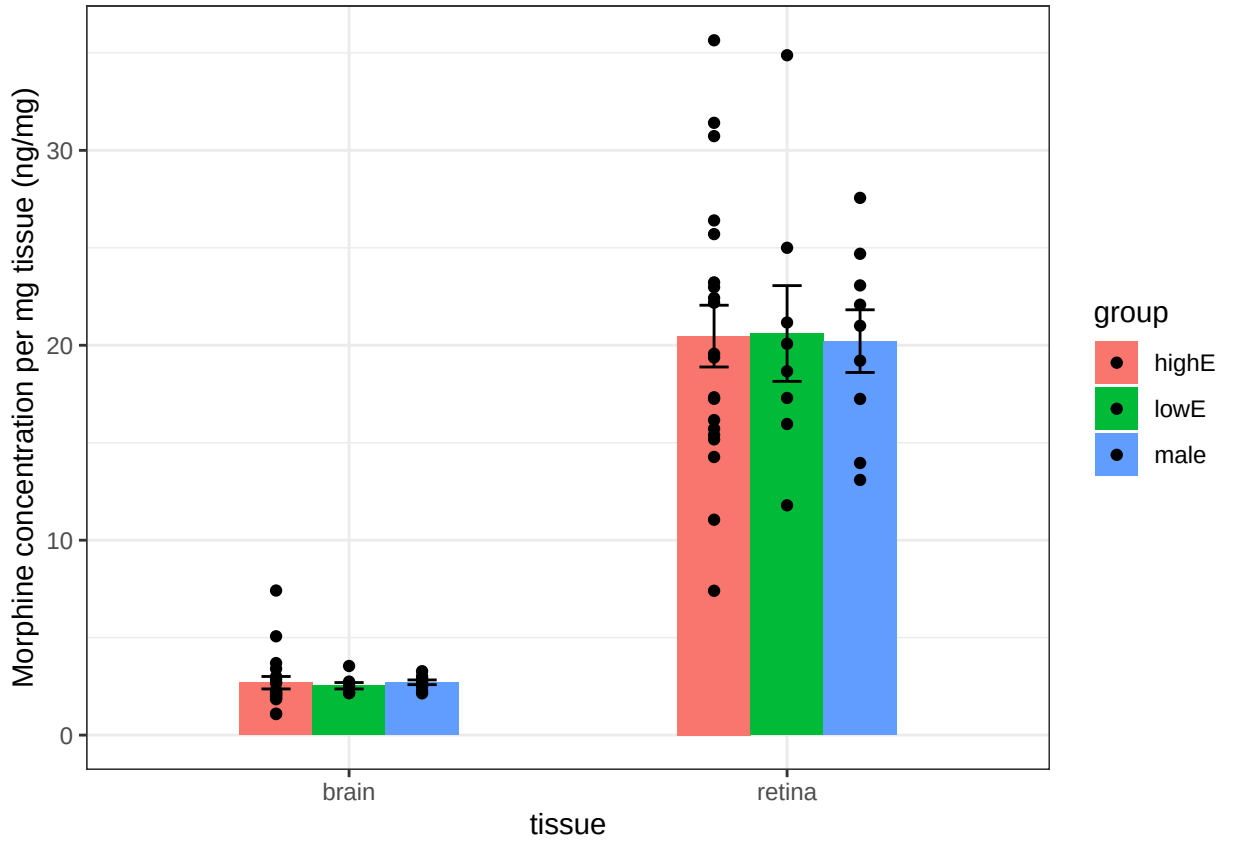



```
ggsave("plot_tissue.svg", plot=plot_tissue, width=5, height =4)
```

```
plot_stage <- ggplot() +
  geom_bar(data=SumStat_sexdiff, aes(x=tissue, y=mean_conc_wt, fill=group), stat="identity", position=po
  geom_errorbar(data=SumStat_sexdiff, aes(x=tissue,ymin=mean_conc_wt - se_conc_wt, ymax=mean_conc_wt + s
  geom_point(data=sex_diff, 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: Ignoring unknown aesthetics: fill
```

```
plot_stage
```

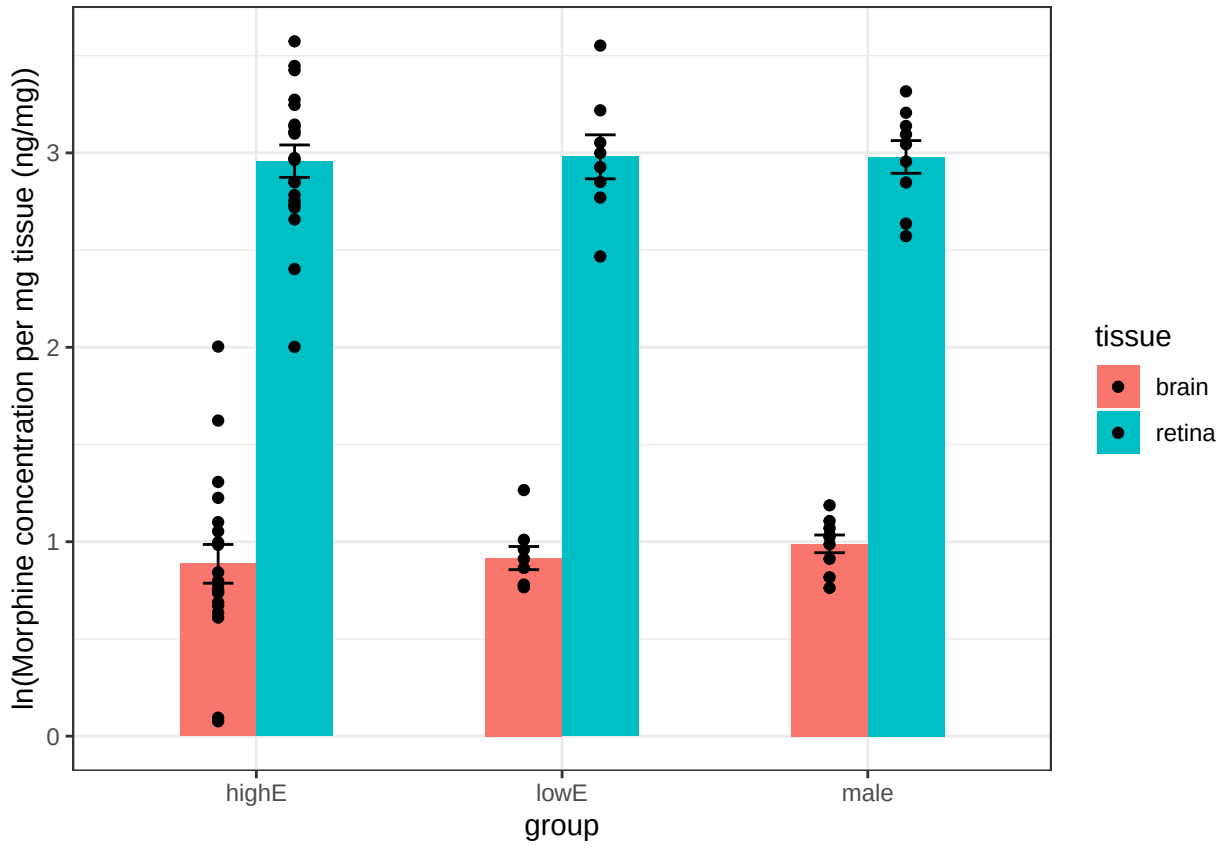



```
ggsave("plot_stage.svg", plot=plot_stage, width=5, height =4)

plot_tissueL <- ggplot() +
  geom_bar(data=SumStat_sexdiff, aes(x=group, y=log_conc, fill=tissue), stat="identity", position=posit
  geom_errorbar(data=SumStat_sexdiff, 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: Ignoring unknown aesthetics: fill
```

```
plot_tissueL
```

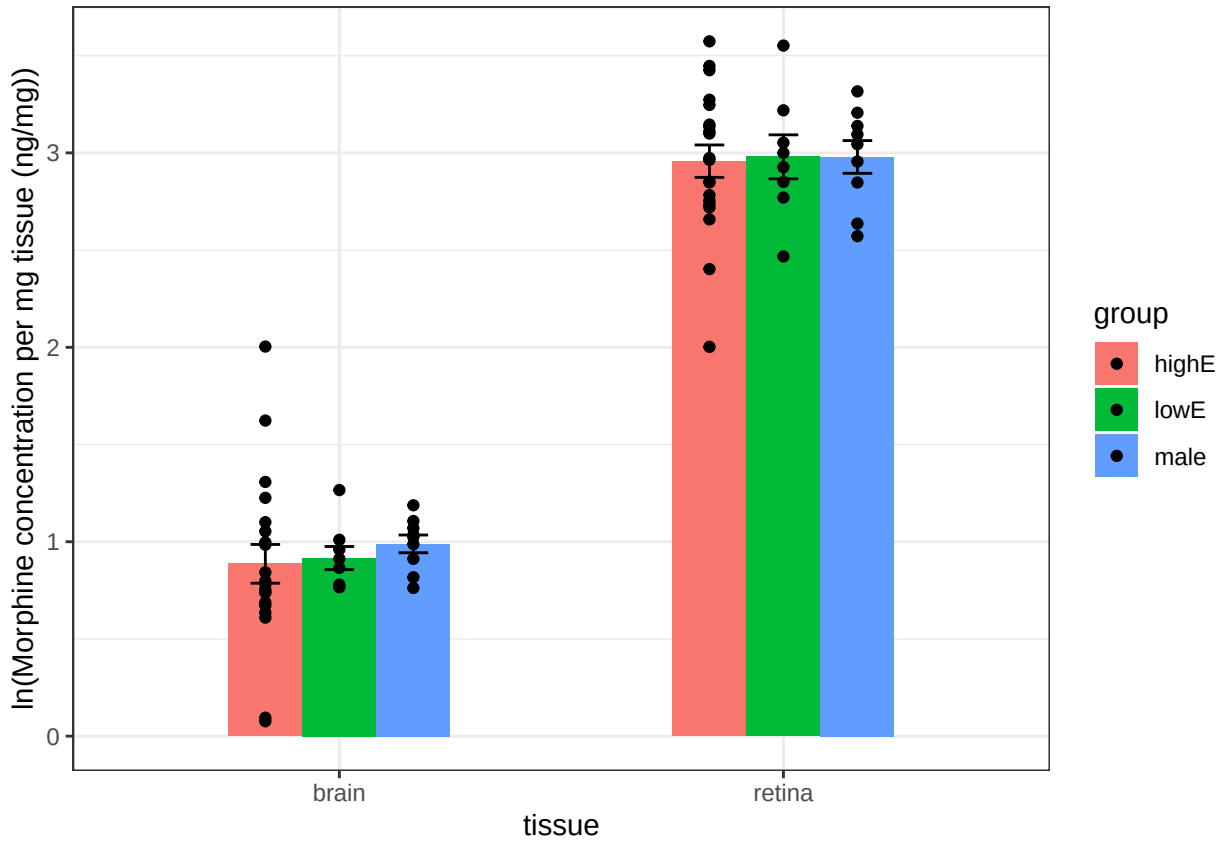



```
ggsave("plot_tissueL.svg", plot=plot_tissueL, width=5, height =4)

plot_stageL <- ggplot() +
  geom_bar(data=SumStat_sexdiff, aes(x=tissue, y=log_conc, fill=group), stat="identity", position=posit
  geom_errorbar(data=SumStat_sexdiff, 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: Ignoring unknown aesthetics: fill
```

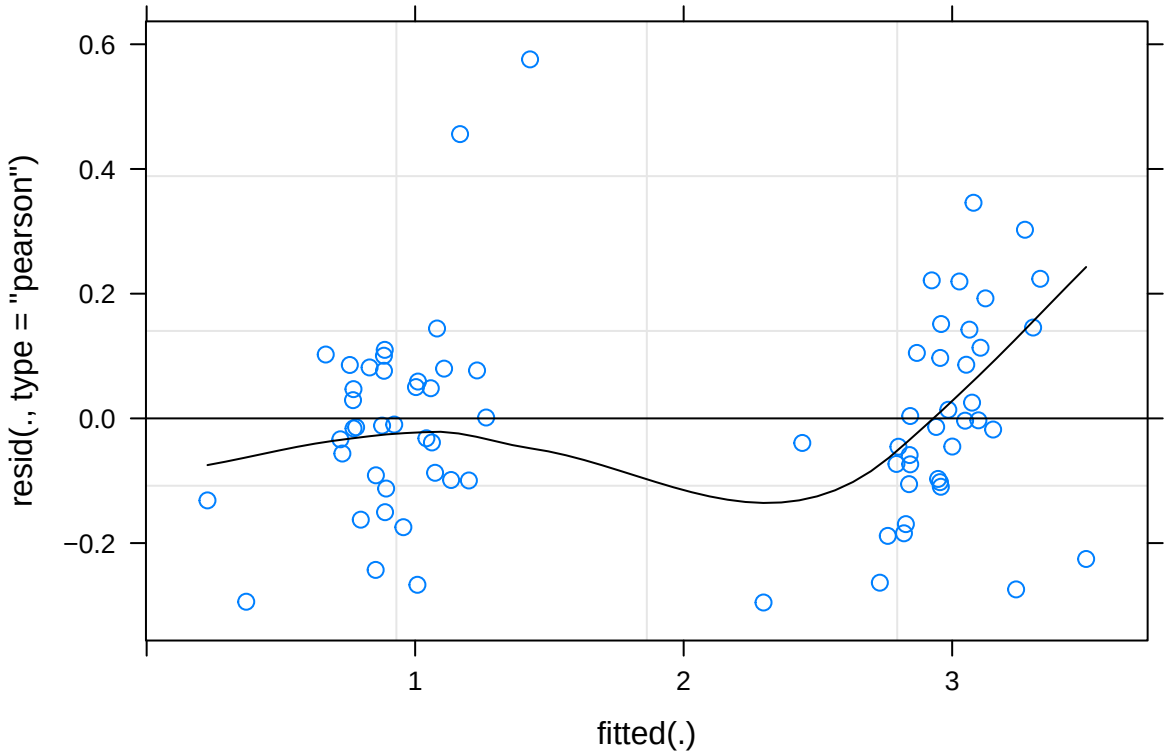
```
plot_stageL
```



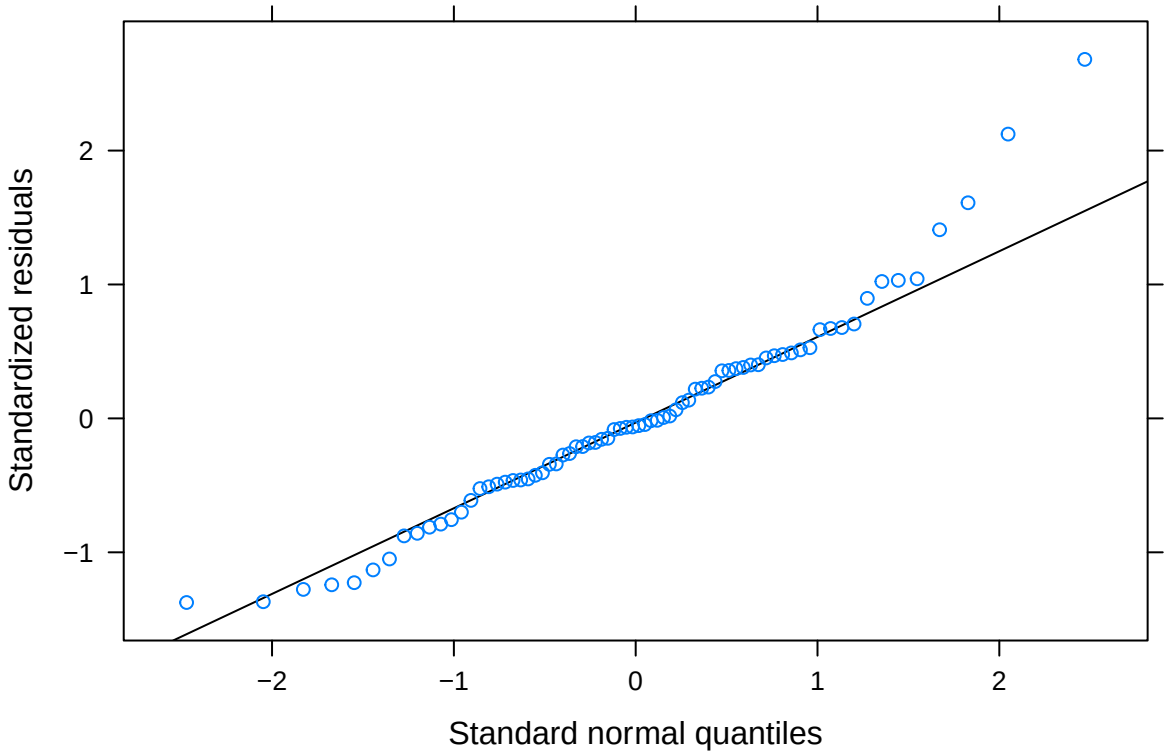

```
ggsave("plot_stageL.svg", plot=plot_stageL, width=5, height =4)
```

Analysis for differences between groups and tissues

```
#sex_diff$stage <- as.factor(sex_diff$stage)
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)
```

```
## Analysis of Variance Table
##              npar Sum Sq Mean Sq   F value
## group           2  0.012   0.006    0.1287
## tissue          1 77.725  77.725 1685.2241
## group:tissue    2  0.021   0.011    0.2313
```

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

```
## $emmeans
## group = highE:
##  tissue emmean     SE   df lower.CL upper.CL
##  brain   0.886 0.0766 49.7    0.732     1.04
##  retina  2.957 0.0766 49.7    2.803     3.11
##
## group = lowE:
##  tissue emmean     SE   df lower.CL upper.CL
##  brain   0.916 0.1212 49.7    0.673     1.16
##  retina  2.980 0.1212 49.7    2.736     3.22
##
## group = male:
##  tissue emmean     SE   df lower.CL upper.CL
##  brain   0.989 0.1142 49.7    0.760     1.22
##  retina  2.979 0.1142 49.7    2.750     3.21
```

```
##
## Degrees-of-freedom method: kenward-roger
## Results are given on the log (not the response) scale.
## Confidence level used: 0.95
##
## $contrasts
## group = highE:
##  contrast       estimate     SE df t.ratio p.value
##  brain - retina    -2.07 0.0679 34 -30.495  <.0001
##
## group = lowE:
##  contrast       estimate     SE df t.ratio p.value
##  brain - retina    -2.06 0.1074 34 -19.218  <.0001
##
## group = male:
##  contrast       estimate     SE df t.ratio p.value
##  brain - retina    -1.99 0.1012 34 -19.657  <.0001
##
## Degrees-of-freedom method: kenward-roger
## Results are given on the log (not the response) scale.
```

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

```
## $emmeans
## tissue = brain:
##  group emmean     SE   df lower.CL upper.CL
##  highE  0.886 0.0766 49.7    0.732     1.04
##  lowE   0.916 0.1212 49.7    0.673     1.16
##  male   0.989 0.1142 49.7    0.760     1.22
##
## tissue = retina:
##  group emmean     SE   df lower.CL upper.CL
##  highE  2.957 0.0766 49.7    2.803     3.11
##  lowE   2.980 0.1212 49.7    2.736     3.22
##  male   2.979 0.1142 49.7    2.750     3.21
##
## Degrees-of-freedom method: kenward-roger
## Results are given on the log (not the response) scale.
## Confidence level used: 0.95
##
## $contrasts
## tissue = brain:
##  contrast     estimate    SE   df t.ratio p.value
##  highE - lowE  -0.0296 0.143 49.7  -0.207  0.9767
##  highE - male  -0.1027 0.138 49.7  -0.746  0.7371
##  lowE - male   -0.0730 0.167 49.7  -0.439  0.8996
##
## tissue = retina:
##  contrast     estimate    SE   df t.ratio p.value
##  highE - lowE  -0.0223 0.143 49.7  -0.156  0.9867
##  highE - male  -0.0217 0.138 49.7  -0.158  0.9863
##  lowE - male    0.0006 0.167 49.7   0.004  1.0000
##
## 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))
```

```
##
##  Shapiro-Wilk normality test
##
## data:  resid(lmer_diff)
## W = 0.95906, p-value = 0.01723
```

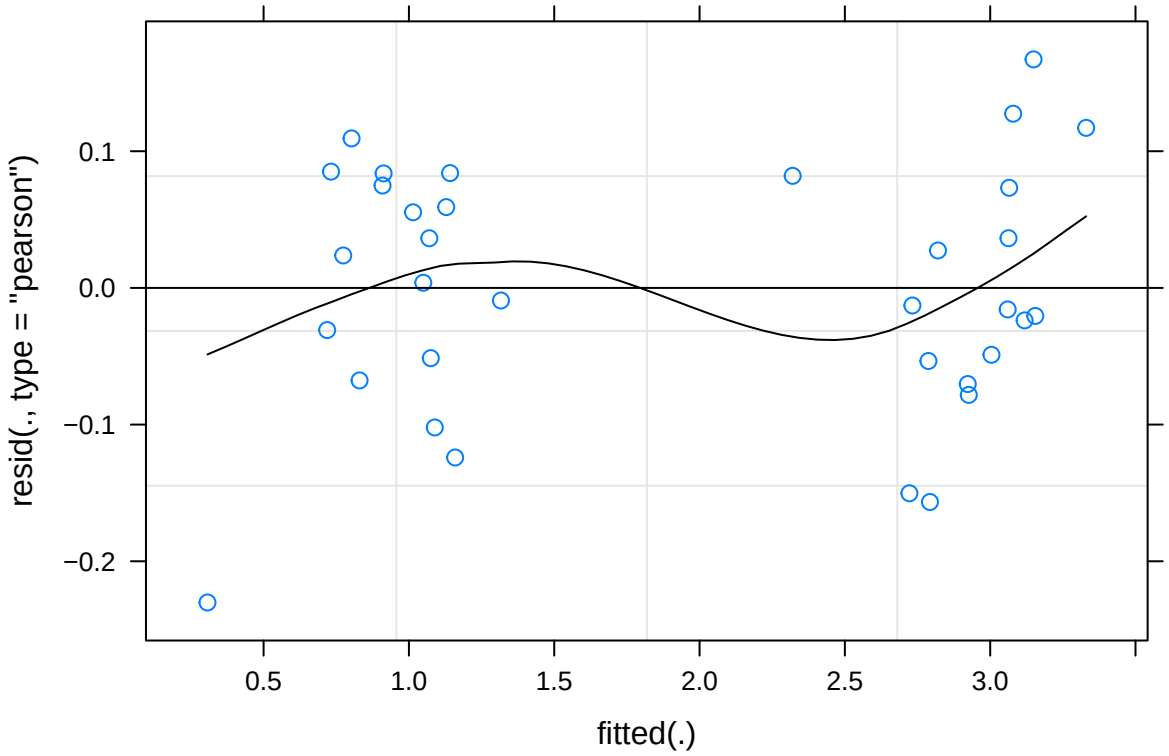
Sex differences analysis exclusively for animals that were littermates

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

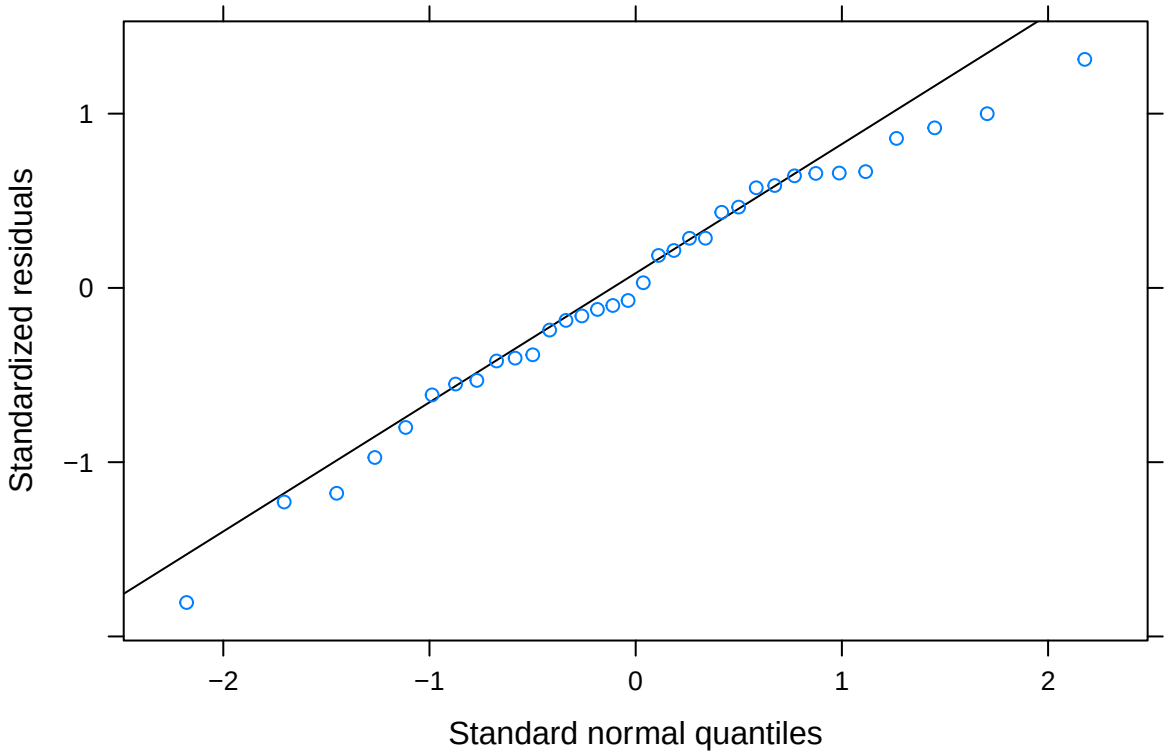
```
##    animal raw_conc tissue_weight body_wt  dose     stage group tissue   conc_wt
## 1      1M  56.5895          20.1    27.2 20.60      male  male  brain  2.815398
## 2      2M  22.0798          10.3    30.1 18.60      male  male  brain  2.143670
## 3      3M  36.7582          13.7    28.7 19.50      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
## 1   1.03510364
```

```
## 2   0.76251927
## 3   0.98696550
## 4   1.18750820
## 5   1.02387158
## 6   0.91209145
## 7   1.06959390
## 8   1.10652958
## 9   0.81715567
## 22  0.98420171
## 23  0.07678345
## 24  1.30740610
## 25  1.05331667
## 26  0.68801134
## 27  1.22571158
## 28  0.99666893
## 29  0.79778243
## 38  3.31641605
## 39  2.84763474
## 40  3.20655929
## 41  3.09467449
## 42  3.13855122
## 43  2.63613572
## 44  2.95538793
## 45  3.04451241
## 46  2.57186868
## 59  2.85244970
## 60  2.40241695
## 61  3.44720842
## 62  3.09938407
## 63  2.71947489
## 64  3.13465734
## 65  2.84810947
## 66  2.73405902
```

```
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)
```

```
## Analysis of Variance Table
##              npar Sum Sq Mean Sq   F value
## group           1  0.007   0.007    0.4345
## tissue          1 34.037  34.037 2093.6731
## group:tissue    1  0.001   0.001    0.0716
```

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

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

```
## $contrasts
## group = highE:
##  contrast       estimate     SE df t.ratio p.value
##  brain - retina    -2.01 0.0638 15 -31.583  <.0001
##
## group = male:
##  contrast       estimate     SE df t.ratio p.value
##  brain - retina    -1.99 0.0601 15 -33.109  <.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 = brain:
##  group emmean     SE   df lower.CL upper.CL
##  highE  0.891 0.1002 18.3    0.681     1.10
##  male   0.989 0.0945 18.3    0.791     1.19
##
## tissue = retina:
##  group emmean     SE   df lower.CL upper.CL
##  highE  2.905 0.1002 18.3    2.694     3.12
##  male   2.979 0.0945 18.3    2.781     3.18
##
## Degrees-of-freedom method: kenward-roger
## Results are given on the log (not the response) scale.
## Confidence level used: 0.95
##
## $contrasts
## tissue = brain:
##  contrast     estimate    SE   df t.ratio p.value
##  highE - male  -0.0978 0.138 18.3  -0.710  0.4866
##
## tissue = retina:
##  contrast     estimate    SE   df t.ratio p.value
##  highE - male  -0.0744 0.138 18.3  -0.540  0.5958
##
## 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
```

Sex differences visualizations exclusively for animals that were littermates

```
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.
```

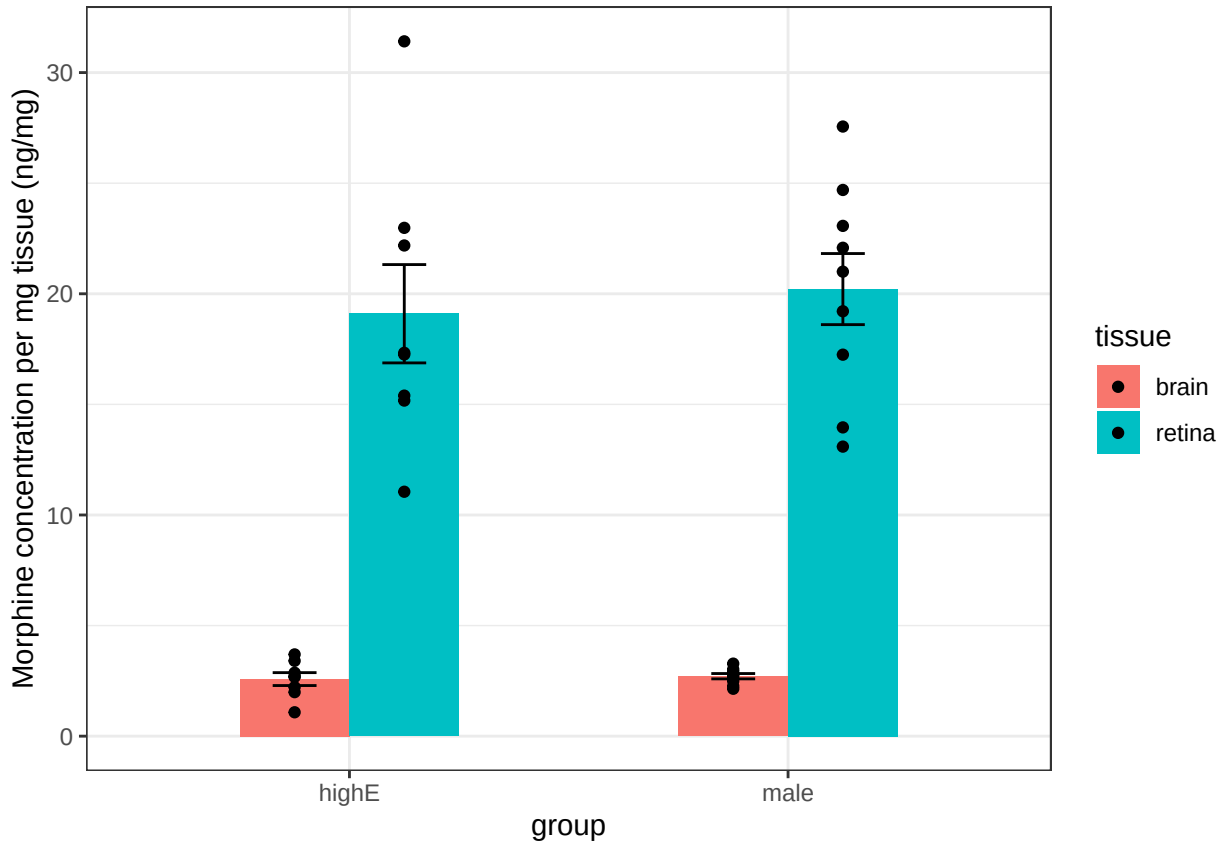
```
SumStat_sexlitt
```

```
## # A tibble: 4 x 13
## # Groups:   tissue [2]
##   tissue group     n mean_conc_wt sd_conc_wt se_conc_wt log_conc sd_conc_log
##   <chr>  <chr> <int>        <dbl>      <dbl>      <dbl>    <dbl>       <dbl>
## 1 brain  highE     8         2.58      0.825      0.292    0.891       0.386
## 2 brain  male      9         2.71      0.362      0.121    0.989       0.137
## 3 retina highE     8        19.1       6.29       2.22     2.90        0.318
## 4 retina male      9        20.2       4.82       1.61     2.98        0.252
## # ... with 5 more variables: se_conc_log <dbl>, mean_wt <dbl>, sd_wt <dbl>,
## #   mean_dose <dbl>, sd_dose <dbl>
```

```
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: Ignoring unknown aesthetics: fill
```

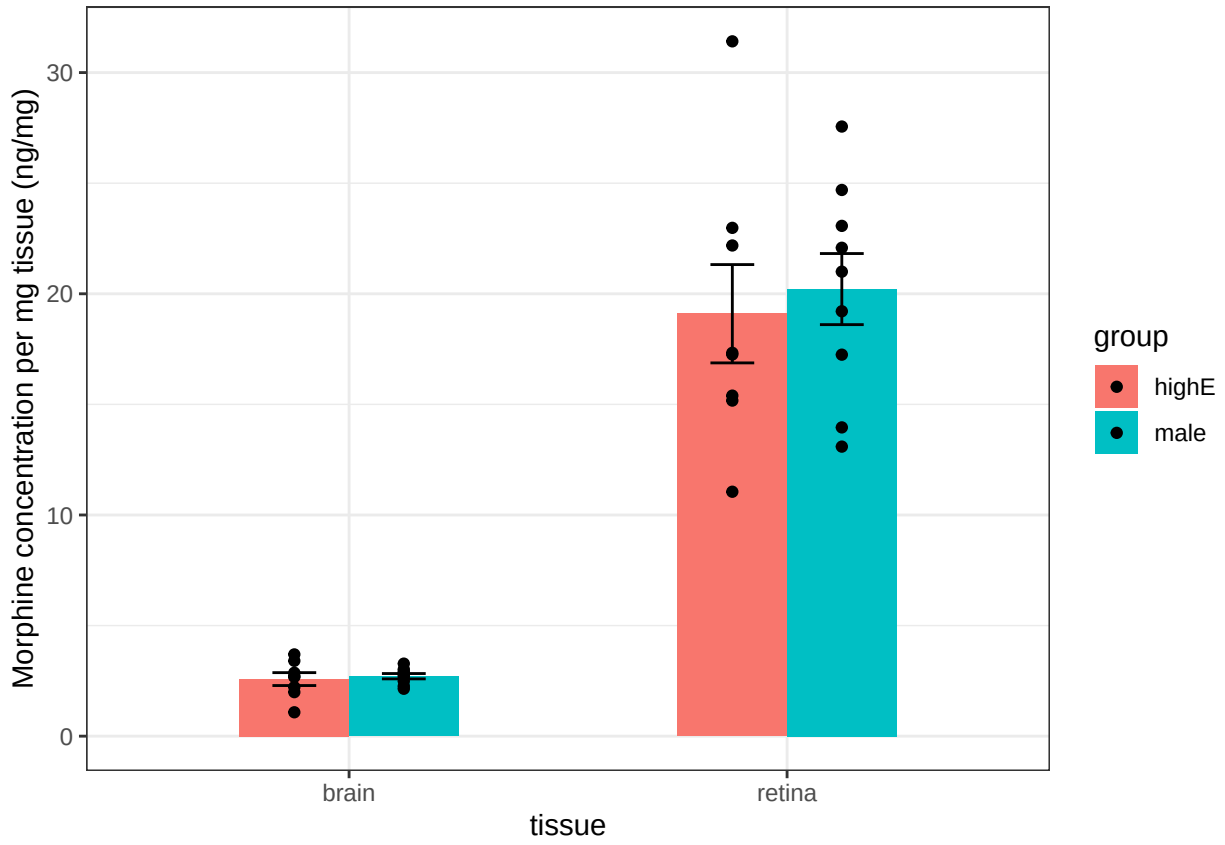
```
plot_tissue_litt
```




```
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: Ignoring unknown aesthetics: fill
```

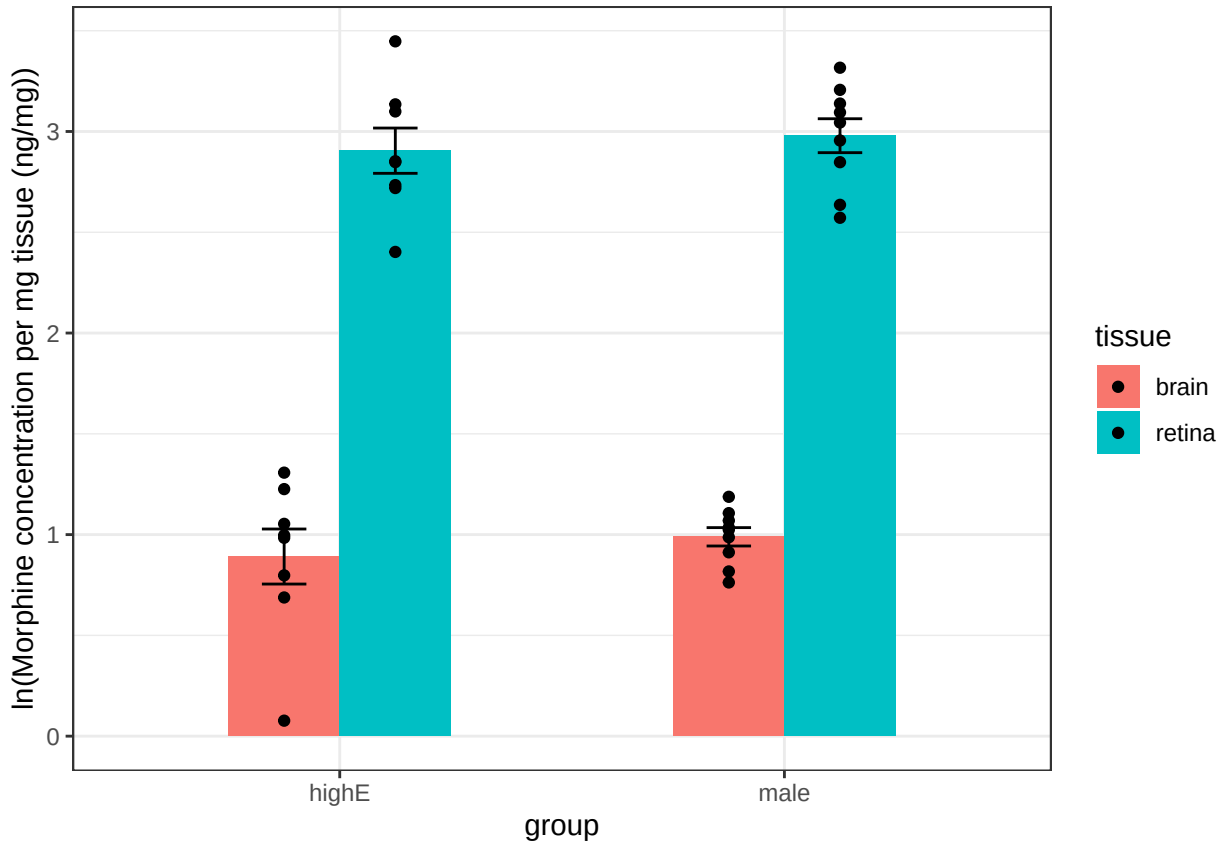
```
plot_stage_litt
```




```
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: Ignoring unknown aesthetics: fill
```

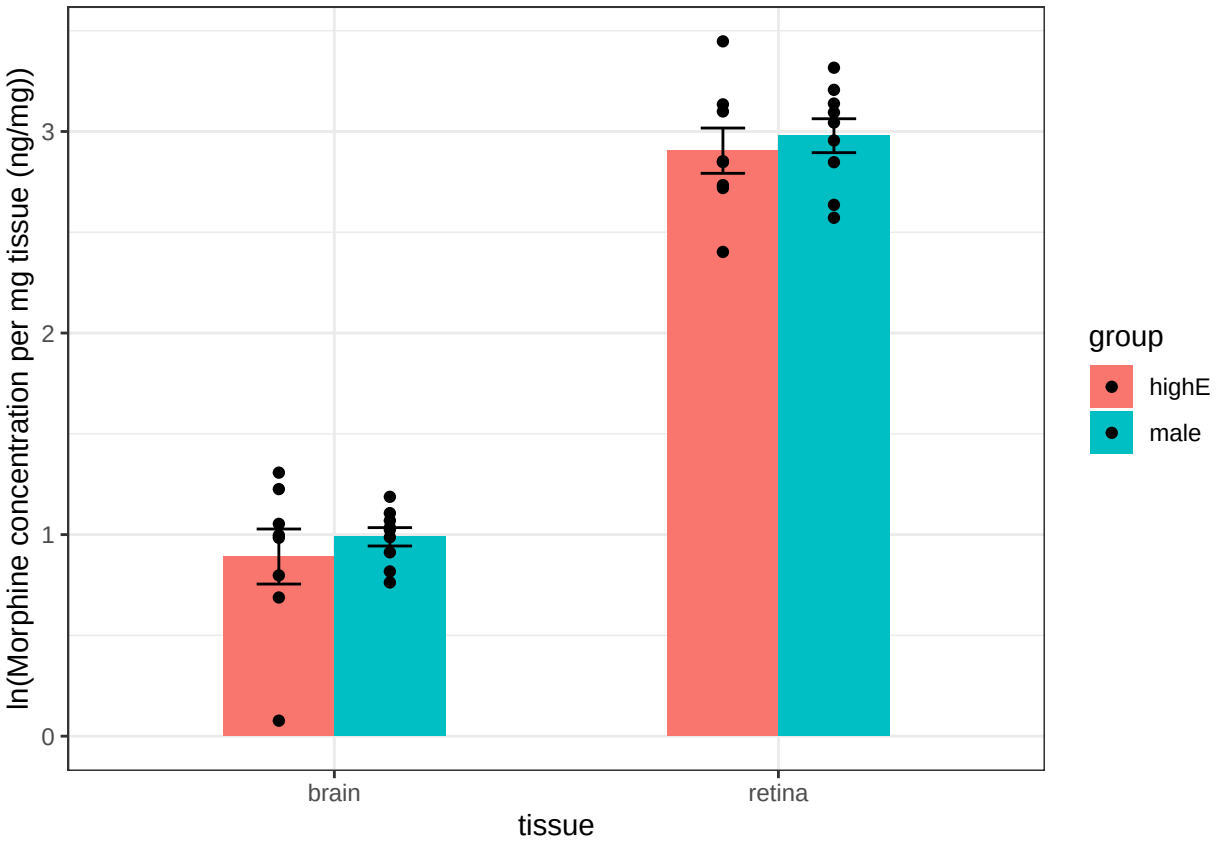
```
plot_tissueL_litt
```




```
plot_stageL_litt <- ggplot() +
  geom_bar(data=SumStat_sexlitt, aes(x=tissue, y=log_conc, fill=group), stat="identity", position=positi
  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: Ignoring unknown aesthetics: fill
```

```
plot_stageL_litt
```



Comparisons between estrus stages

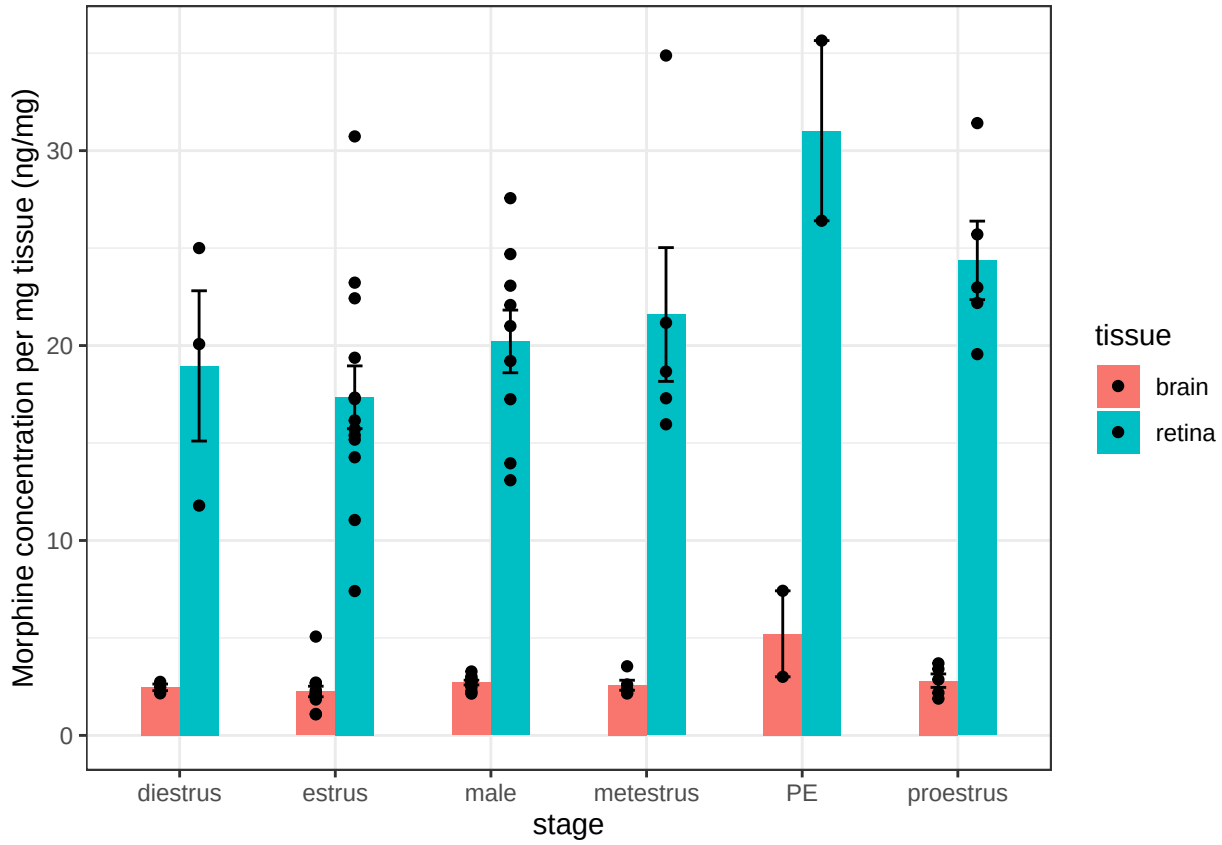
```
SumStat_sexstage <- dplyr::summarise(group_by(sex_diff, tissue, stage),
n = n(),
mean_conc_wt = mean(conc_wt),
sd_conc_wt = sd(conc_wt),
se_conc_wt = sd_conc_wt/sqrt(n),
log_conc = mean(log(conc_wt)),
sd_conc_log = sd(log(conc_wt)),
se_conc_log = sd_conc_log/sqrt(n),
mean_wt = mean(body_wt),
sd_wt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

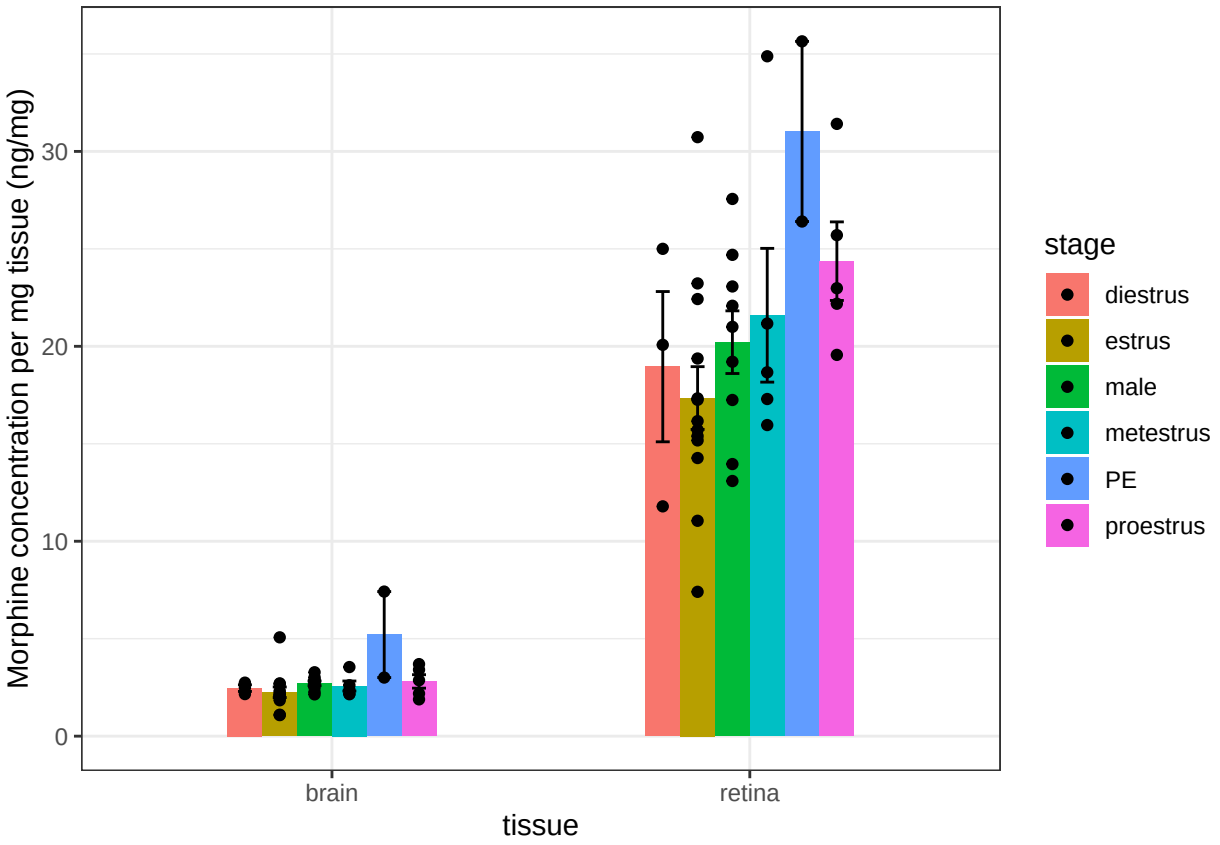
```
plot_stage_tissue
```



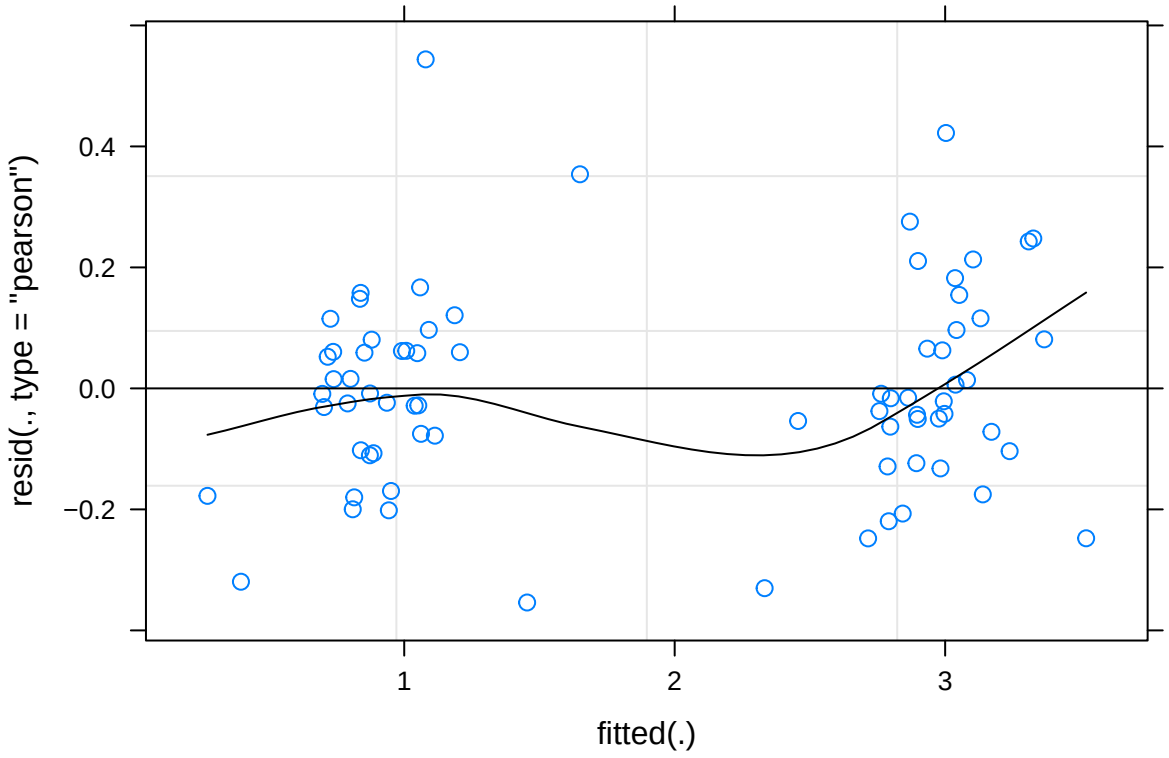

```
plot_stages <- ggplot() +
  geom_bar(data=SumStat_sexstage, aes(x=tissue, y=mean_conc_wt, fill=stage), stat="identity", position=
  geom_errorbar(data=SumStat_sexstage, aes(x=tissue,ymin=mean_conc_wt - se_conc_wt, ymax=mean_conc_wt +
  geom_point(data=sex_diff, aes(x=tissue, y=conc_wt, fill=stage),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

```
## Warning: Ignoring unknown aesthetics: fill
```

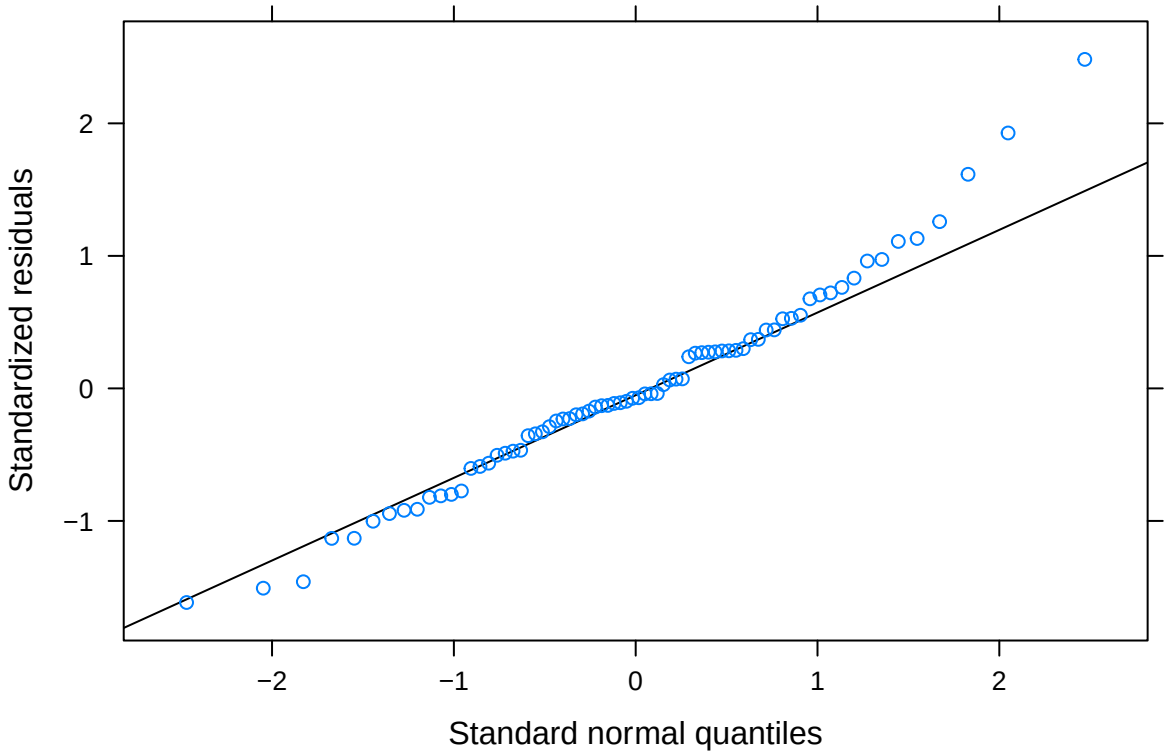
```
plot_stages
```




```
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)
```

```
## Analysis of Variance Table
##              npar Sum Sq Mean Sq   F value
## stage           5  0.789   0.158    3.2863
## tissue          1 77.725  77.725 1619.6736
## stage:tissue    5  0.102   0.020    0.4244
```

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

```
## $emmeans
## stage = diestrus:
##  tissue emmean     SE   df lower.CL upper.CL
##  brain   0.897 0.1753 50.4    0.545    1.249
##  retina  2.895 0.1753 50.4    2.543    3.247
##
## stage = estrus:
##  tissue emmean     SE   df lower.CL upper.CL
##  brain   0.740 0.0842 50.4    0.571    0.909
##  retina  2.800 0.0842 50.4    2.631    2.969
##
## stage = male:
##  tissue emmean     SE   df lower.CL upper.CL
##  brain   0.989 0.1012 50.4    0.786    1.192
```

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