

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)

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

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 : Factor w/ 3 levels "male","lowE",...: 1 1 1 1 1 1 1 1 1 3 ...
## $ tissue : Factor w/ 2 levels "retina","brain": 2 2 2 2 2 2 2 2 2 2 ...
## $ 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 ...
```

```
#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.
```

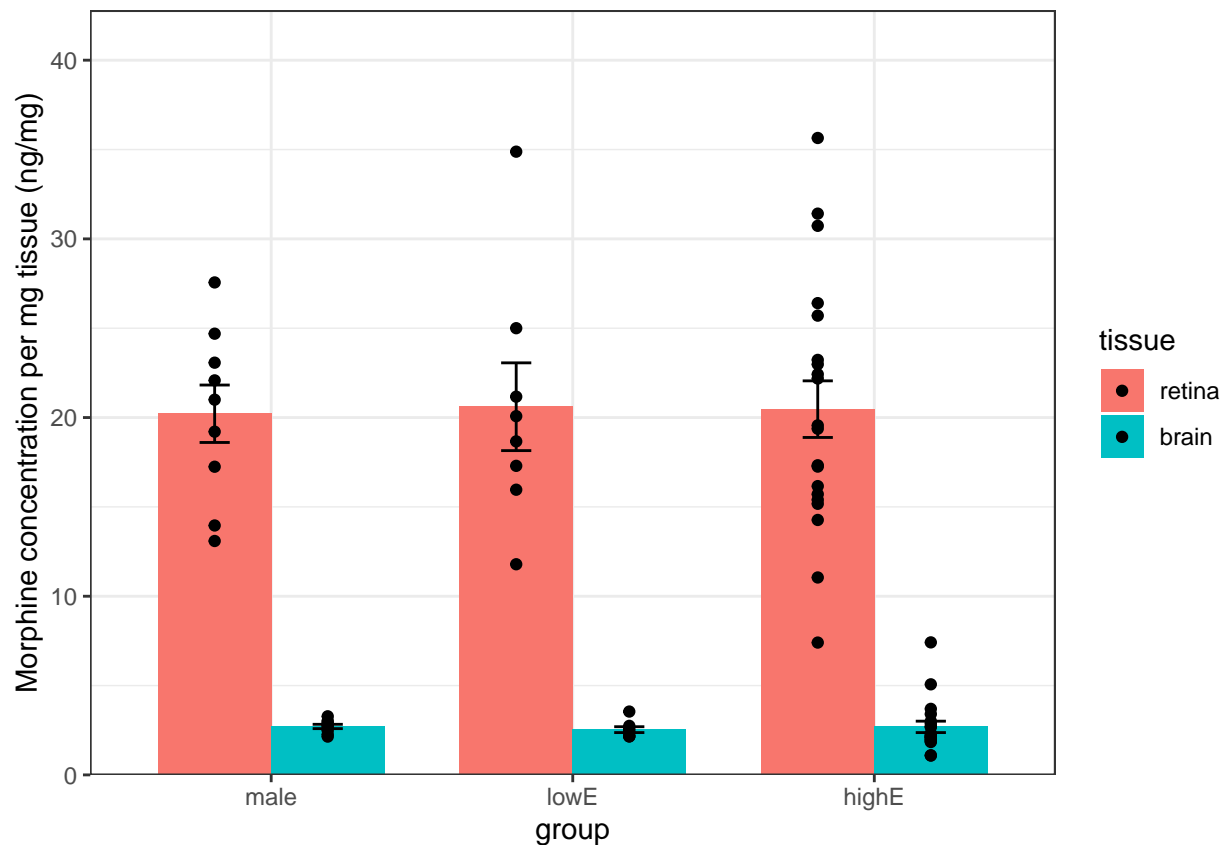
```
SumStat_sexdiff
```

```
## # A tibble: 6 x 13
## # Groups:   tissue [2]
##   tissue group      n mean_conc~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>
## 1 retina male      9      20.2    4.82    1.61    2.98    0.252  0.0842   26.9
## 2 retina lowE      8      20.6    6.95    2.46    2.98    0.320  0.113   20.8
## 3 retina highE     20      20.5    7.08    1.58    2.96    0.373  0.0834   21.4
## 4 brain  male      9       2.71   0.362   0.121   0.989   0.137  0.0456   26.9
## 5 brain  lowE      8       2.53   0.465   0.164   0.916   0.168  0.0595   20.8
## 6 brain  highE     20       2.69   1.43    0.320   0.886   0.445  0.0996   21.4
## # ... 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
## # i Use `colnames()` to see all variable names
```

```
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=.2) +
  geom_point(data=sex_diff, aes(x=group, y=conc_wt, fill=tissue),
    position=position_dodge(width=0.75)) +
  scale_y_continuous(name="Morphine concentration per mg tissue (ng/mg)",
    expand = expansion(mult = c(0, 0.2))) +
  theme_bw()
```

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

```
plot_tissue
```

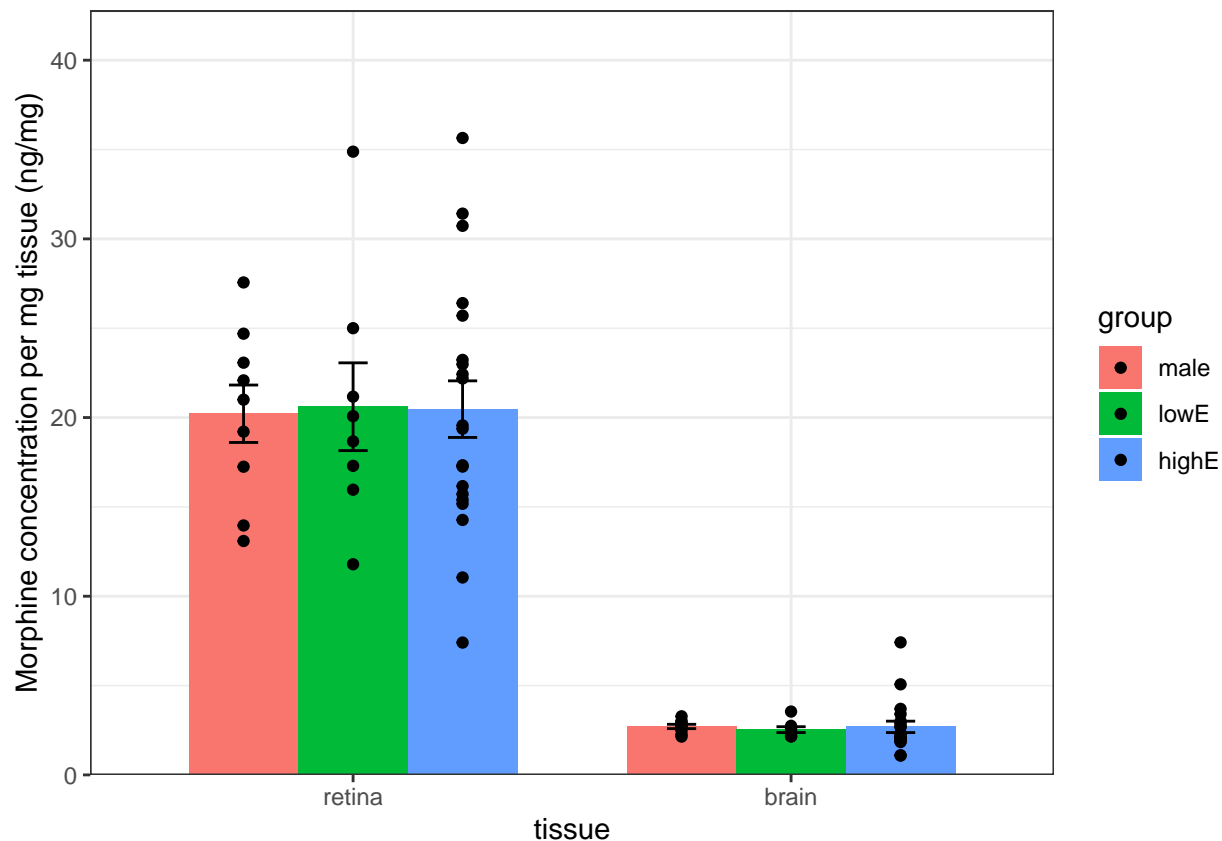


```
#ggsave("../figures/conc_tissue.svg", plot=plot_tissue, width=6, height =4)
```

```
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=.2) +
  geom_point(data=sex_diff, aes(x=tissue, y=conc_wt, fill=group),
    position=position_dodge(width=0.75)) +
  scale_y_continuous(name="Morphine concentration per mg tissue (ng/mg)",
    expand = expansion(mult = c(0, 0.2))) +
  theme_bw()
```

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

```
plot_stage
```

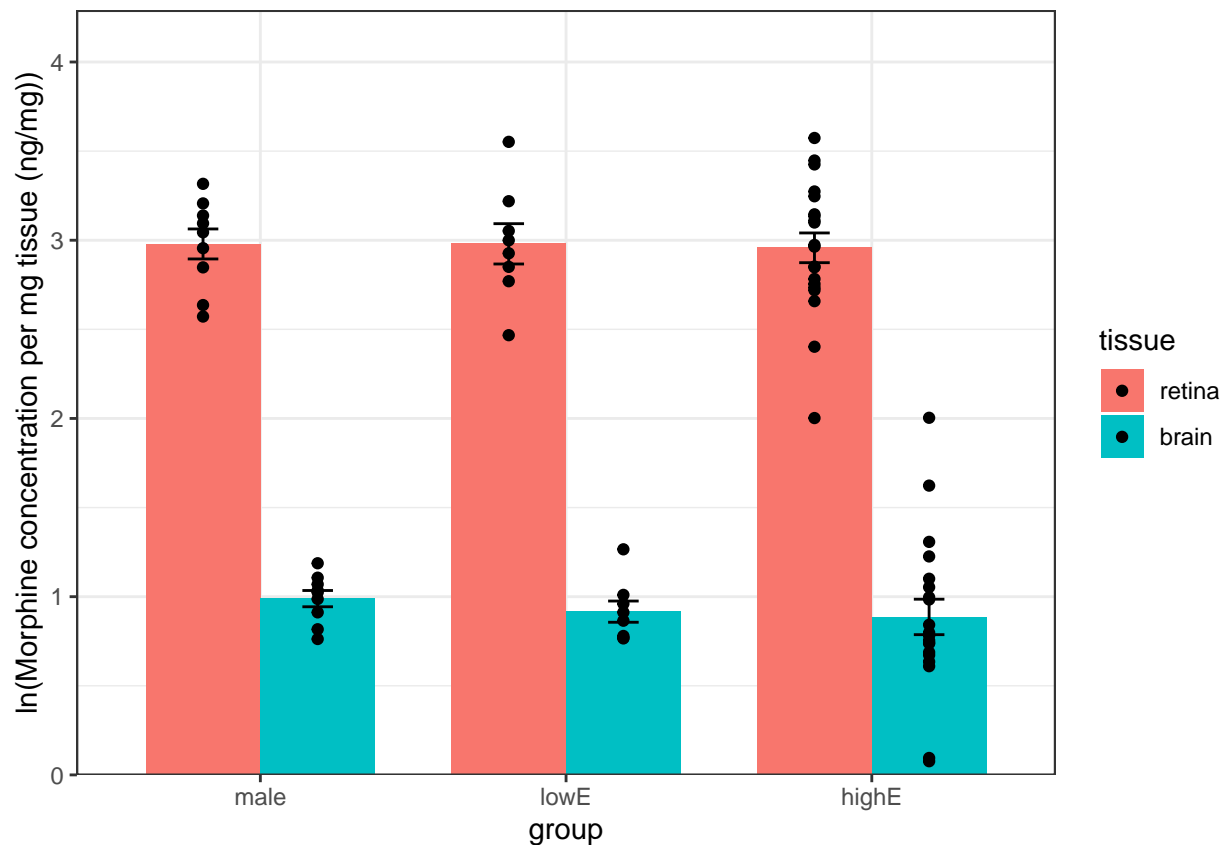


```
#ggsave("../figures/conc_stage.svg", plot=plot_stage, width=6, height =4)
```

```
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=.2) +
  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 concentration per mg tissue (ng/mg))",
    expand = expansion(mult = c(0, 0.2))) +
  theme_bw()
```

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

```
plot_tissueL
```

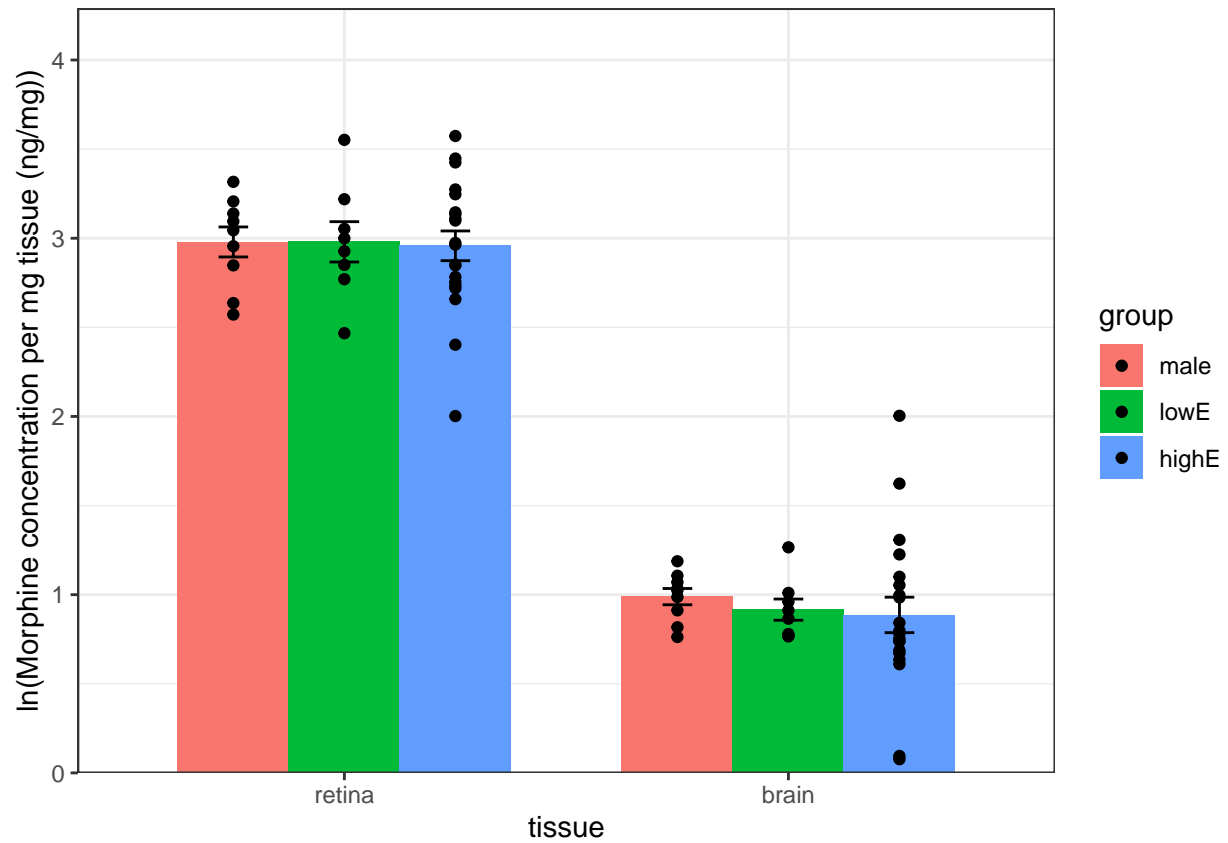


```
#ggsave("../figures/conc_tissue_log.svg", plot=plot_tissueL, width=6, height =4)
```

```
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=.2) +
  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 concentration per mg tissue (ng/mg))",
    expand = expansion(mult = c(0, 0.2))) +
  theme_bw()
```

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

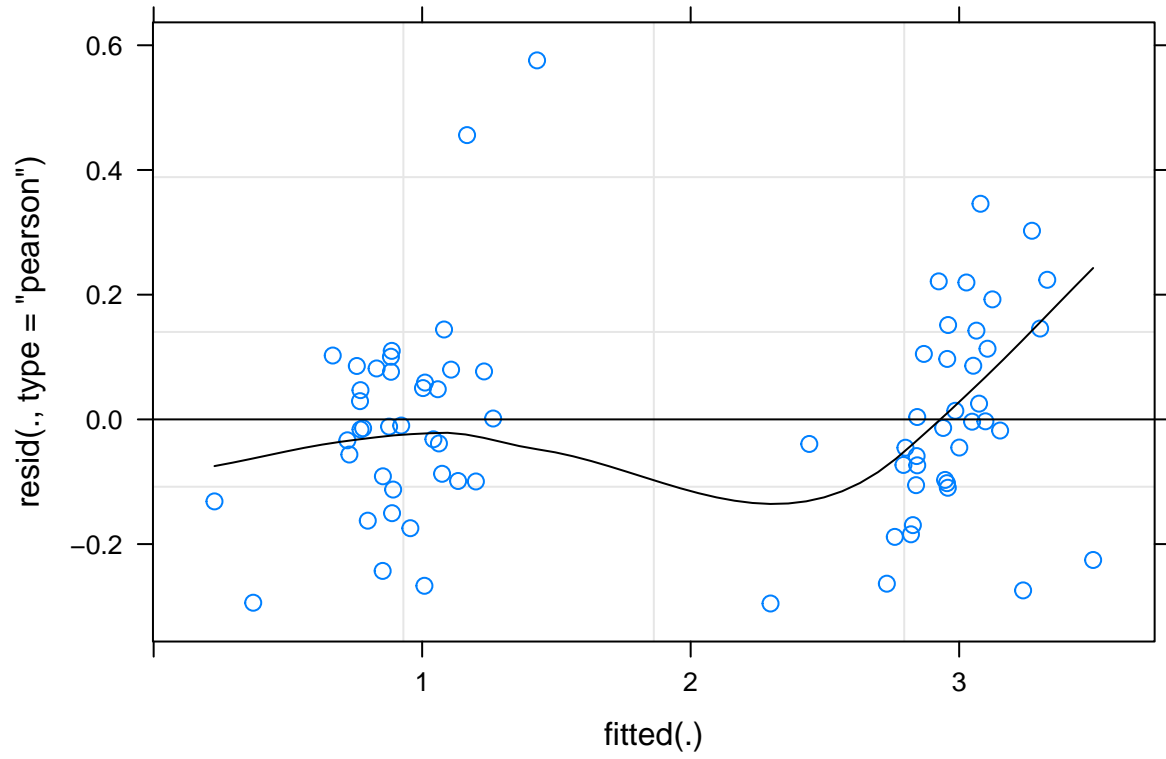
```
plot_stageL
```



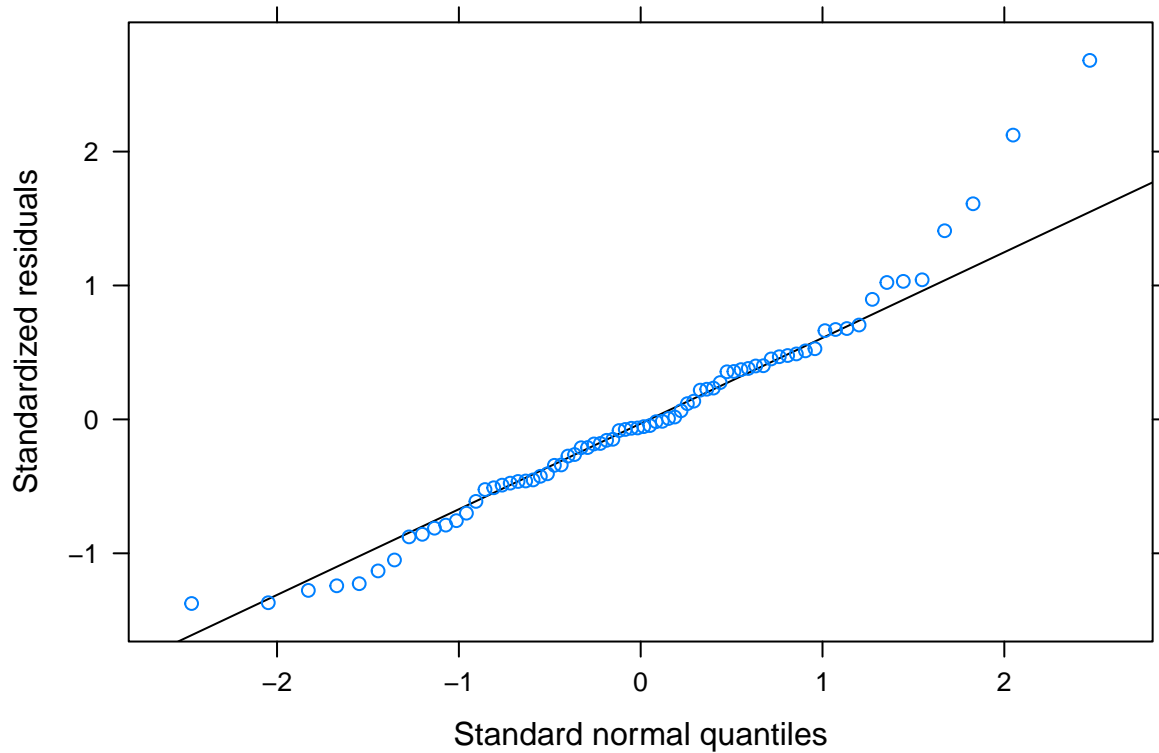
```
#ggsave("../figures/conc_stage_log.svg", plot=plot_stageL, width=6, height =4)
```

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="3")
```

```
## Analysis of Deviance Table (Type III Wald chisquare tests)
##
## Response: log(conc_wt)
##           Chisq Df Pr(>Chisq)
## (Intercept)  680.1794  1    <2e-16 ***
## group         0.0379  2     0.9812
## tissue       386.3966  1    <2e-16 ***
## group:tissue   0.4625  2     0.7935
## ---
## 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))
```

```
##
## 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
```

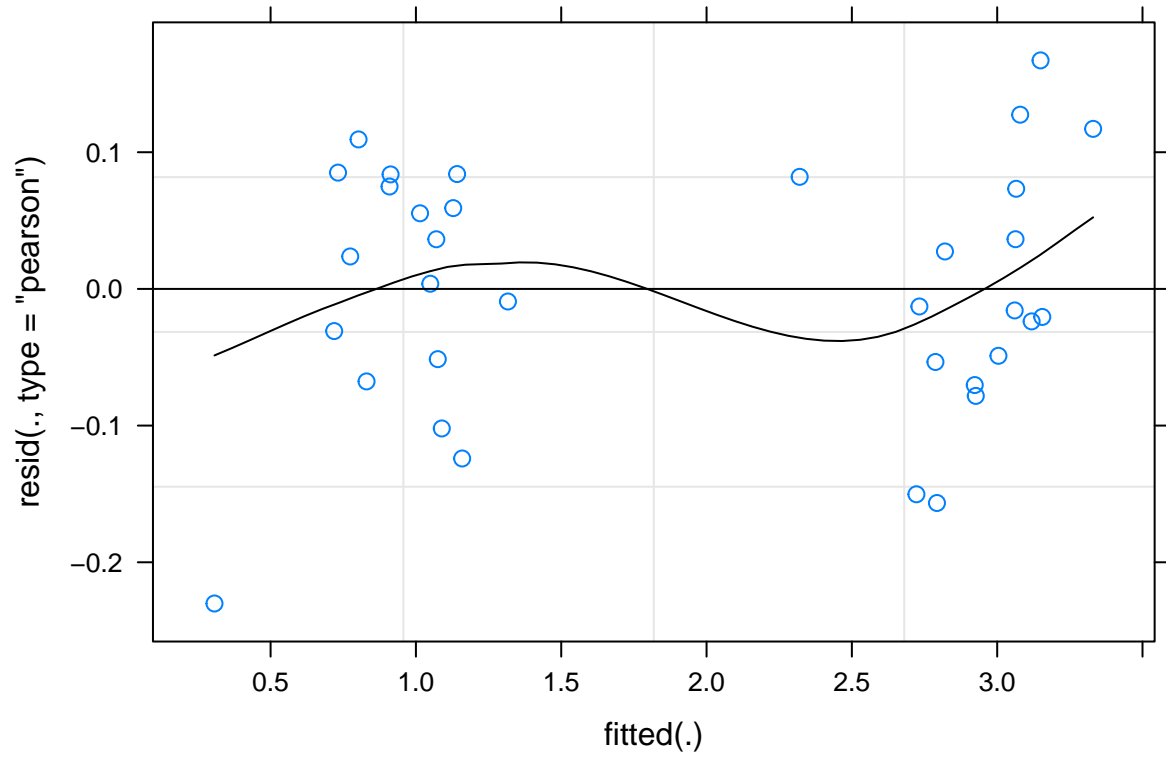
	animal	raw_conc	tissue_weight	body_wt	dose	stage	group	tissue	conc_wt
## 1	1M	56.5895	20.1	27.2	20.60	male	male	brain	2.815398
## 2	2M	22.0798	10.3	30.1	18.60	male	male	brain	2.143670
## 3	3M	36.7582	13.7	28.7	19.50	male	male	brain	2.683080
## 4	4M	49.5114	15.1	25.8	21.70	male	male	brain	3.278901
## 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

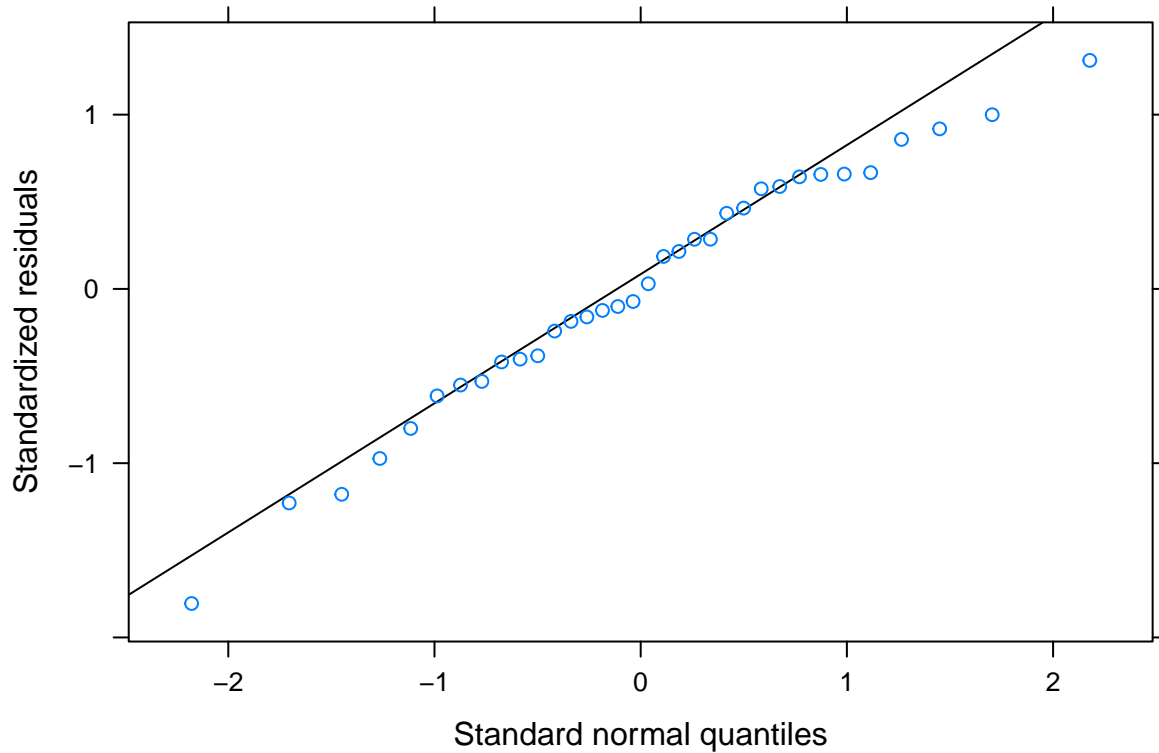
log_conc_wt

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 = 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
```

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.
```

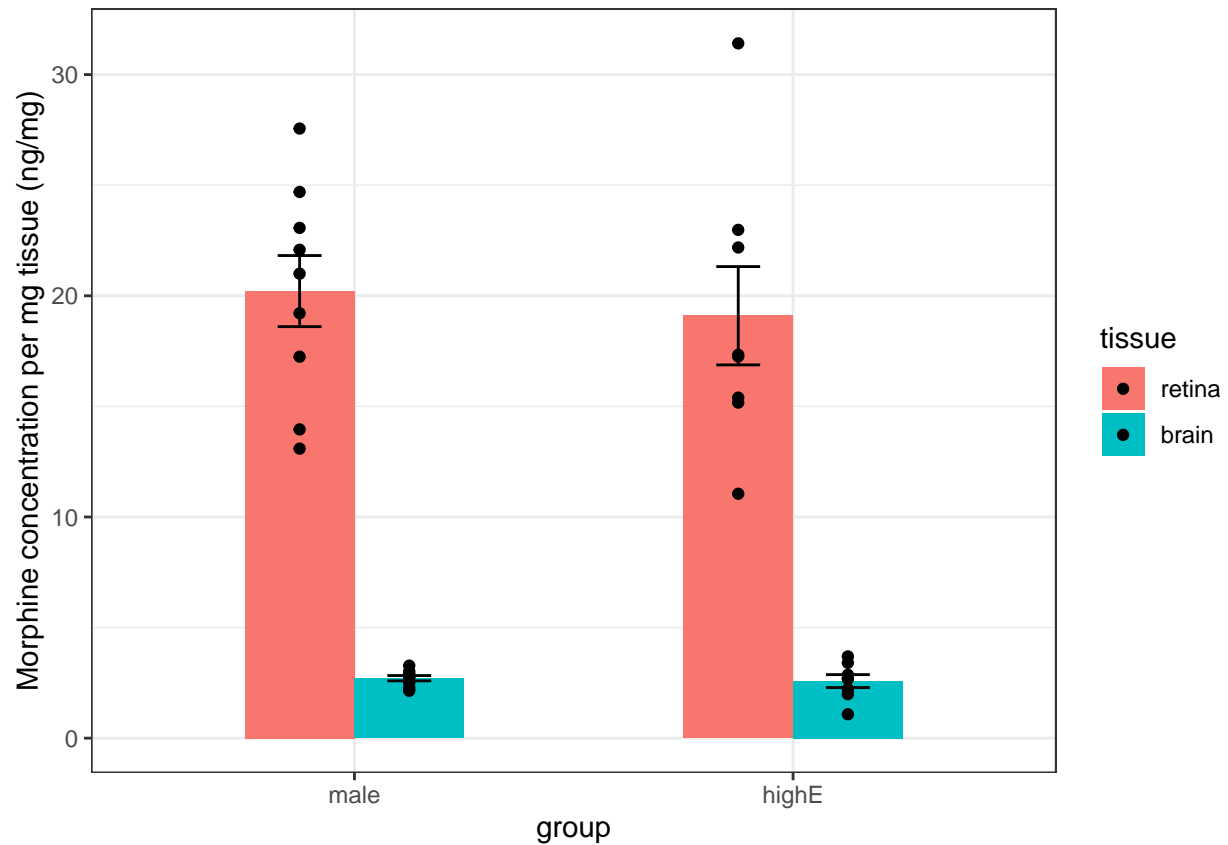
```
SumStat_sex1itt
```

```
## # 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
## # i Use `colnames()` to see all variable names
```

```
plot_tissue_litt <- ggplot() +
  geom_bar(data=SumStat_sex1itt, aes(x=group, y=mean_conc_wt, fill=tissue), stat="identity", position=position_dodge(width=0.5)) +
  geom_errorbar(data=SumStat_sex1itt, aes(x=group, ymin=mean_conc_wt - se_conc_wt, ymax=mean_conc_wt + se_conc_wt), position=position_dodge(width=0.5)) +
  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
```

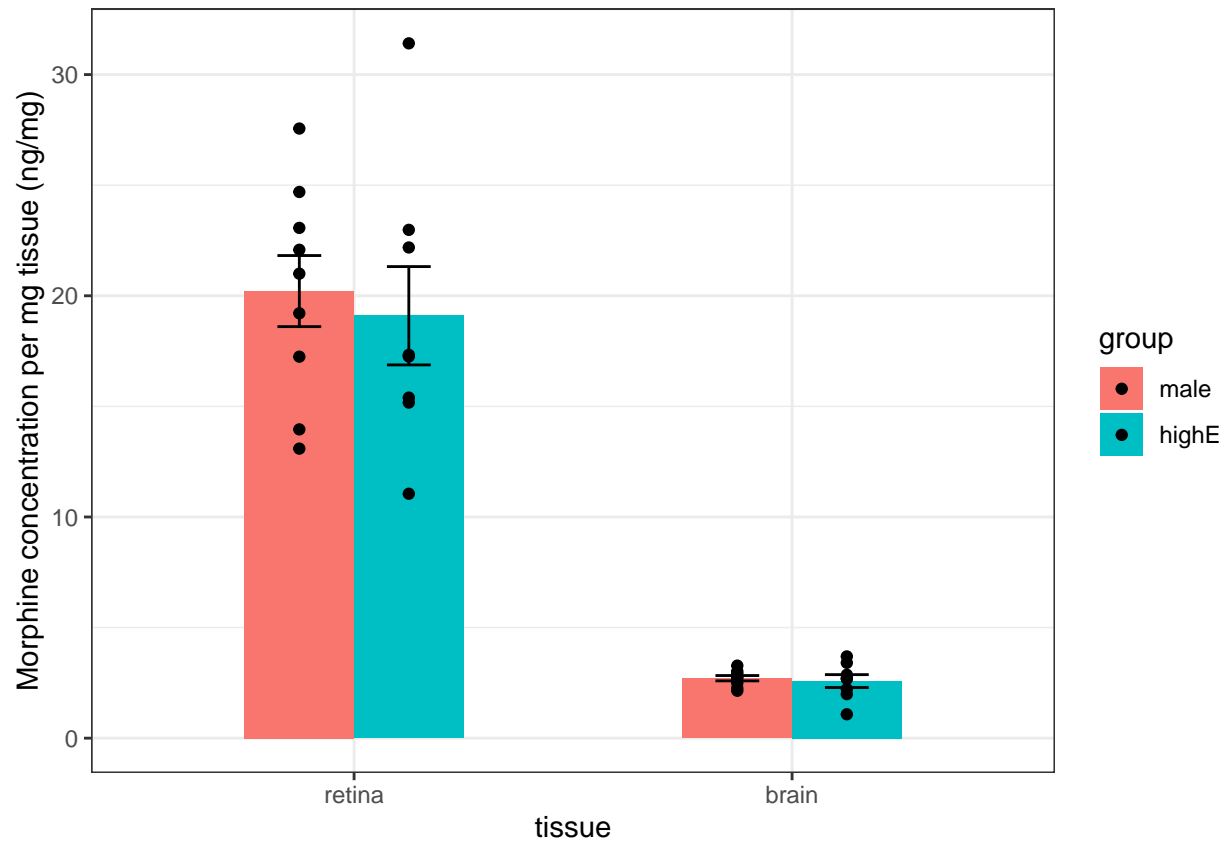
```
plot_tissue_litt
```



```
plot_stage_litt <- ggplot() +
  geom_bar(data=SumStat_sex_litt, aes(x=tissue, y=mean_conc_wt, fill=group), stat="identity", position=position_dodge(width=0.5)) +
  geom_errorbar(data=SumStat_sex_litt, aes(x=tissue, ymin=mean_conc_wt - se_conc_wt, ymax=mean_conc_wt + se_conc_wt), position=position_dodge(width=0.5)) +
  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
```

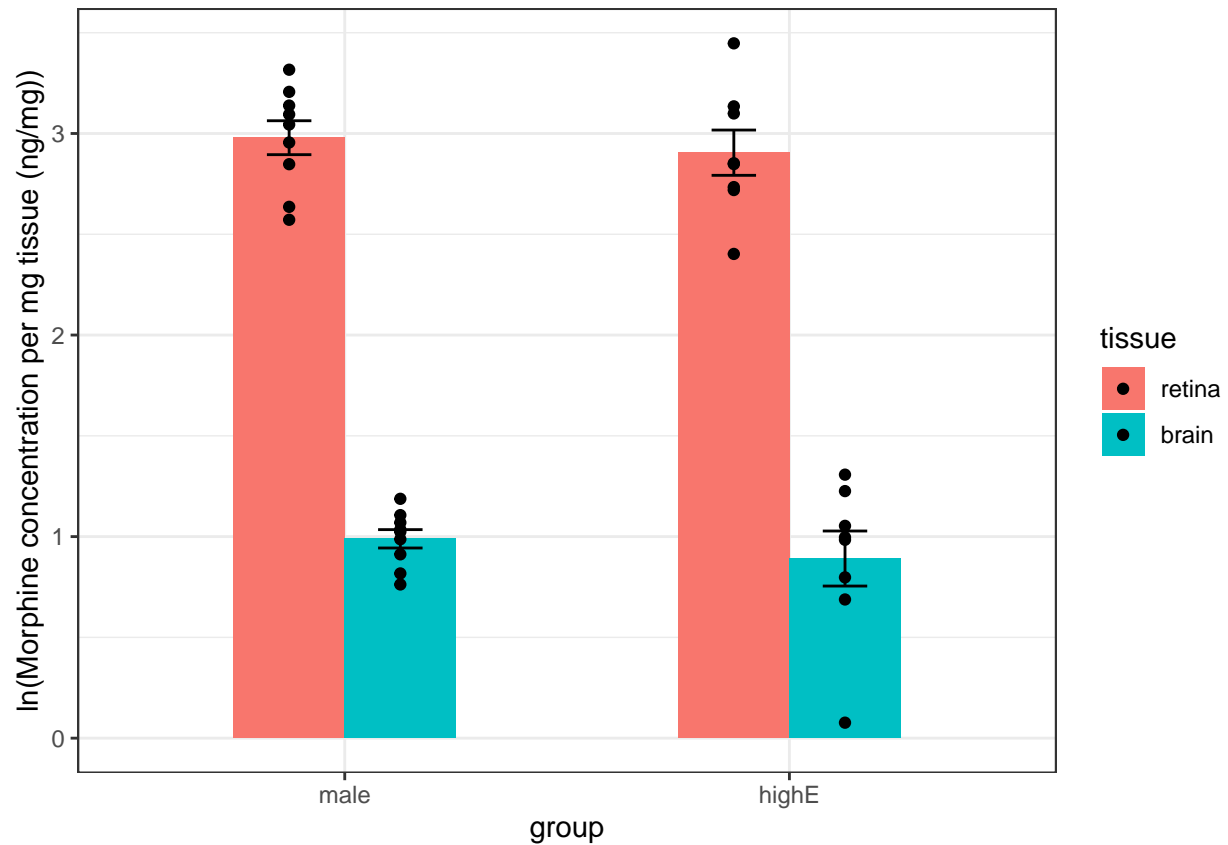
```
plot_stage_litt
```

```
plot_tissueL_litt <- ggplot() +
  geom_bar(data=SumStat_sexLitt, aes(x=group, y=log_conc, fill=tissue), stat="identity", position=position_dodge()) +
  geom_errorbar(data=SumStat_sexLitt, aes(x=group, ymin=log_conc - se_conc_log, ymax=log_conc + se_conc_log), width=0.5) +
  scale_y_continuous(name="ln(Morphine concentration per mg tissue (ng/mg))") + theme_bw()
```

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

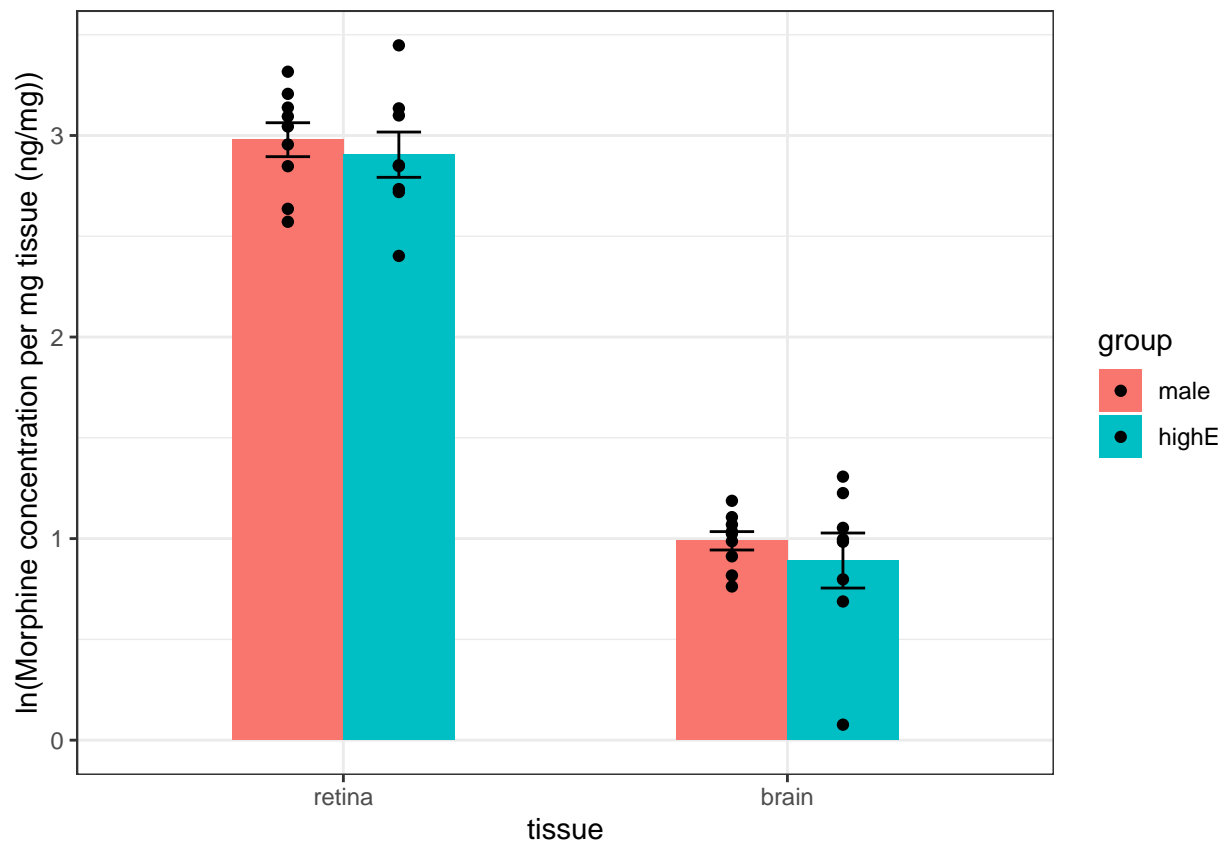
```
plot_tissueL_litt
```



```
plot_stageL_litt <- ggplot() +
  geom_bar(data=SumStat_sexLitt, aes(x=tissue, y=log_conc, fill=group), stat="identity", position=position_dodge()) +
  geom_errorbar(data=SumStat_sexLitt, aes(x=tissue, ymin=log_conc - se_conc_log, ymax=log_conc + se_conc_log), width=.5) +
  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

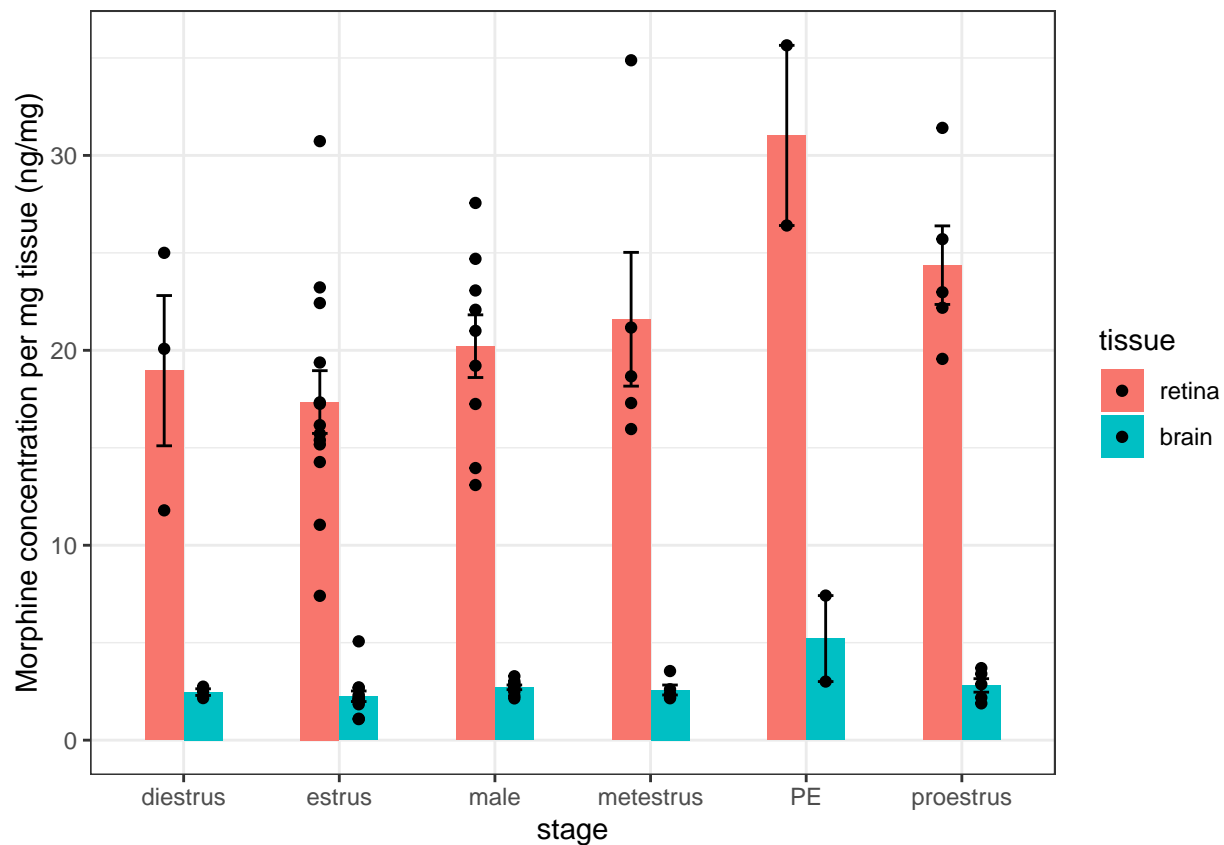
```
SumStat_sexstage <- dplyr::summarise(group_by(sex_diff, tissue, stage),
  n = n(),
  mean_conc_wt = mean(conc_wt),
  sd_conc_wt = sd(conc_wt),
  se_conc_wt = sd_conc_wt/sqrt(n),
  log_conc = mean(log(conc_wt)),
  sd_conc_log = sd(log(conc_wt)),
  se_conc_log = sd_conc_log/sqrt(n),
  mean_wt = mean(body_wt),
  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=position_dodge(width=0.5)) +
  geom_errorbar(data=SumStat_sexstage, aes(x=stage, ymin=mean_conc_wt - se_conc_wt, ymax=mean_conc_wt + se_conc_wt, fill=tissue), position=position_dodge(width=0.5)) +
  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
```

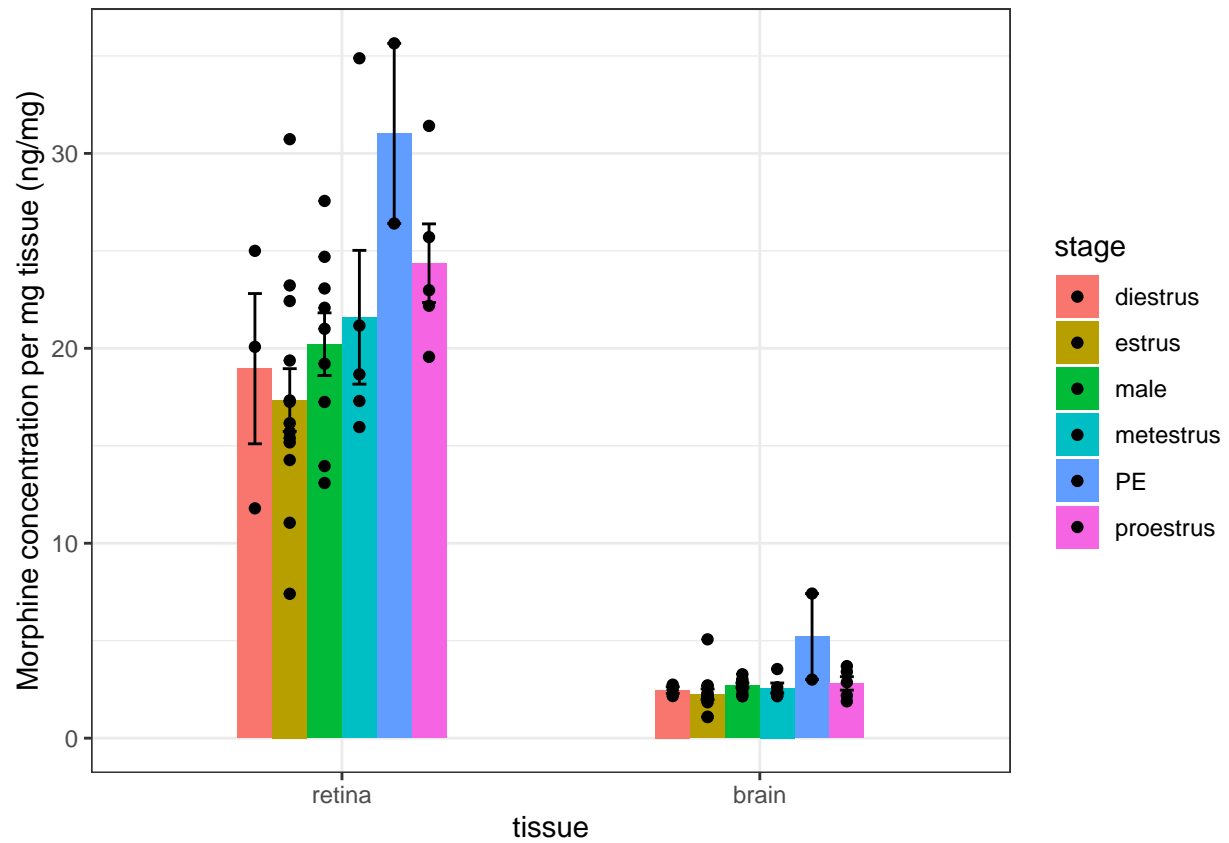
```
plot_stage_tissue
```



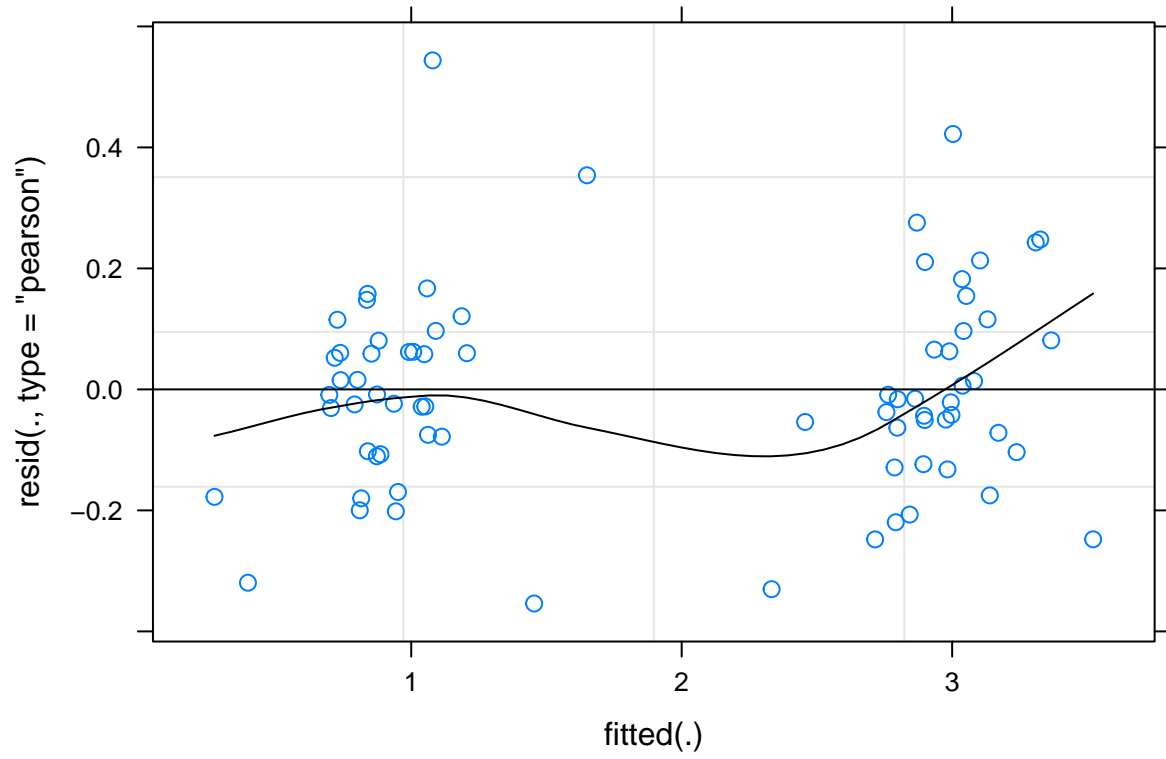
```
plot_stages <- ggplot() +
  geom_bar(data=SumStat_sexstage, aes(x=tissue, y=mean_conc_wt, fill=stage), stat="identity", position=position_dodge(width=0.5)) +
  geom_errorbar(data=SumStat_sexstage, aes(x=tissue, ymin=mean_conc_wt - se_conc_wt, ymax=mean_conc_wt + se_conc_wt, fill=stage), position=position_dodge(width=0.5)) +
  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
```

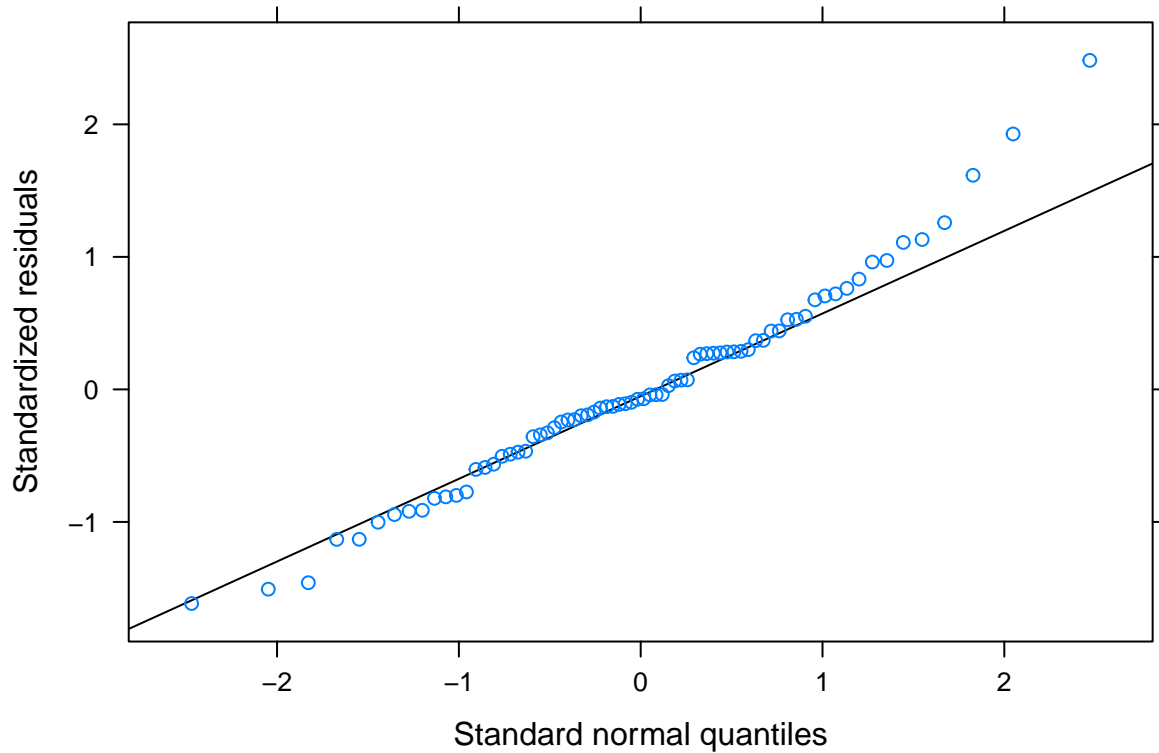
```
plot_stages
```



```
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
##   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
```