

Sleep Bout Analysis

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```
bouts <- read.csv("../data/WT_McKO_combined_bouts.csv",
  fileEncoding = 'UTF-8-BOM')
bouts <- bouts %>% mutate(Time = mdy_hms(Time),
  Sleep.Stage = as.factor(Sleep.Stage),
  Genotype = as.factor(Genotype),
  Transmitter = as.factor(Transmitter))

bouts <- bouts %>% mutate(Hour = hour(Time))
bouts <- bouts %>% mutate(ZT = Time + hours(18))
bouts <- bouts %>% mutate(ZT = hour(ZT))

bouts <- bouts %>% dplyr::mutate(phase = as.factor(ifelse(ZT>11, "dark", "light")))

head(bouts)
```

```
##      Bout Epoch              Time Sleep.Stage Count Duration Genotype
## 1      1  2216 2019-02-10 18:00:00          S     36      360    McKO
## 2      2  2252 2019-02-10 18:06:00          W      2       20    McKO
## 3      3  2254 2019-02-10 18:06:20          S      6       60    McKO
## 4      4  2260 2019-02-10 18:07:20          W      4       40    McKO
## 5      5  2264 2019-02-10 18:08:00          S      4       40    McKO
## 6      6  2268 2019-02-10 18:08:40          W     17      170    McKO
##      Transmitter Day Hour ZT phase
## 1      988823     1   18 12  dark
## 2      988823     1   18 12  dark
## 3      988823     1   18 12  dark
## 4      988823     1   18 12  dark
## 5      988823     1   18 12  dark
## 6      988823     1   18 12  dark
```

```
SumStats_daily <- bouts %>% group_by(Genotype, Transmitter, Sleep.Stage, Day, phase) %>%
  summarise(n_bouts = n(),
    total_duration = sum(Duration),
    min_bout_length = min(Duration),
    mean_bout_length = mean(Duration),
    max_bout_length = max(Duration),
    sd_bout_length = sd(Duration))
```

'summarise()' has grouped output by 'Genotype', 'Transmitter', 'Sleep.Stage', 'Day'. You can override

```
head(SumStats_daily)
```

```
## # A tibble: 6 x 11
## # Groups:   Genotype, Transmitter, Sleep.Stage, Day [3]
##   Genotype Transmitter Sleep.Stage Day phase n_bouts total_duration
##   <fct>     <fct>       <fct>   <int> <fct>   <int>         <int>
## 1 McKO     1050240      P         1 dark     52         1770
## 2 McKO     1050240      P         1 light    86         2990
## 3 McKO     1050240      P         2 dark     37         1290
## 4 McKO     1050240      P         2 light   146         4050
## 5 McKO     1050240      P         3 dark    111         1690
## 6 McKO     1050240      P         3 light   118         3220
## # ... with 4 more variables: min_bout_length <int>, mean_bout_length <dbl>,
## #   max_bout_length <int>, sd_bout_length <dbl>
```

```
SumStats_phase <- SumStats_daily %>% group_by(Genotype, Transmitter, Sleep.Stage, phase) %>%
  summarise(daily_bouts = mean(n_bouts),
            daily_duration = mean(total_duration),
            min_bout = min(min_bout_length),
            mean_bout = mean(mean_bout_length),
            max_bout = max(max_bout_length))
```

'summarise()' has grouped output by 'Genotype', 'Transmitter', 'Sleep.Stage'. You can override using

```
head(SumStats_phase)
```

```
## # A tibble: 6 x 9
## # Groups:   Genotype, Transmitter, Sleep.Stage [3]
##   Genotype Transmitter Sleep.Stage phase daily_bouts daily_duration min_bout
##   <fct>     <fct>       <fct>   <fct>     <dbl>         <dbl>     <int>
## 1 McKO     1050240      P       dark      66.7         1583.        10
## 2 McKO     1050240      P       light     117.         3420         10
## 3 McKO     1050240      S       dark      90.3        15800         10
## 4 McKO     1050240      S       light     169         29763         10
## 5 McKO     1050240      W       dark     108.         25880         10
## 6 McKO     1050240      W       light     138          9907         10
## # ... with 2 more variables: mean_bout <dbl>, max_bout <int>
```

```
SumStats_phase_grps <- SumStats_phase %>% group_by(Genotype, Sleep.Stage, phase) %>%
  summarise(n = n(),
            daily_bout_len = mean(daily_bouts),
            daily_dur = mean(daily_duration),
            avg_bout_length = mean(mean_bout),
            min_bout_length = min(min_bout),
            max_bout_length = max(max_bout))
```

'summarise()' has grouped output by 'Genotype', 'Sleep.Stage'. You can override using the '.groups' a

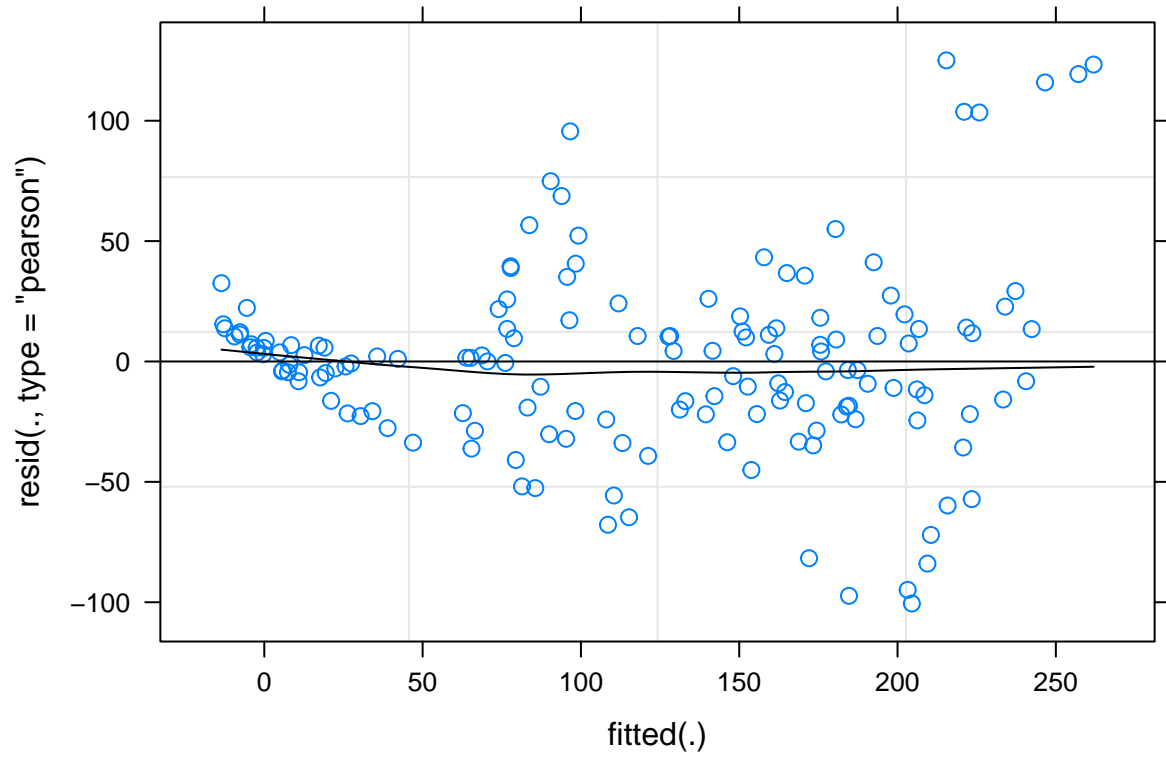
```
SumStats_phase_grps
```

```
## # A tibble: 16 x 9
## # Groups:   Genotype, Sleep.Stage [8]
##   Genotype Sleep.Stage phase      n daily_bout_len daily_dur avg_bout_length
##   <fct>     <fct>      <fct> <int>         <dbl>      <dbl>      <dbl>
## 1 McKO      P          dark     9           83.8       1636.       21.1
## 2 McKO      P          light     9           96.5       3061.       31.0
## 3 McKO      S          dark     9          191.      18047.      111.
## 4 McKO      S          light     9          169.      30235.      191.
## 5 McKO      W          dark     9          222.      23622.      130.
## 6 McKO      W          light     9          146.       9634.       68.8
## 7 McKO      X          dark     9           10.9        109.        10
## 8 McKO      X          light     9            5.72        57.2        10
## 9 WT        P          dark    10           88.3       1881.       26.2
## 10 WT       P          light    10           85.6       3210.       36.8
## 11 WT       S          dark    10          196.      17024.      101.
## 12 WT       S          light    10          174.      28213.      166.
## 13 WT       W          dark    10          232.      24289.      129.
## 14 WT       W          light    10          151.      11502.      81.3
## 15 WT       X          dark    10           17.3        173.        10
## 16 WT       X          light    10            9.32        93.2        10
## # ... with 2 more variables: min_bout_length <int>, max_bout_length <int>
```

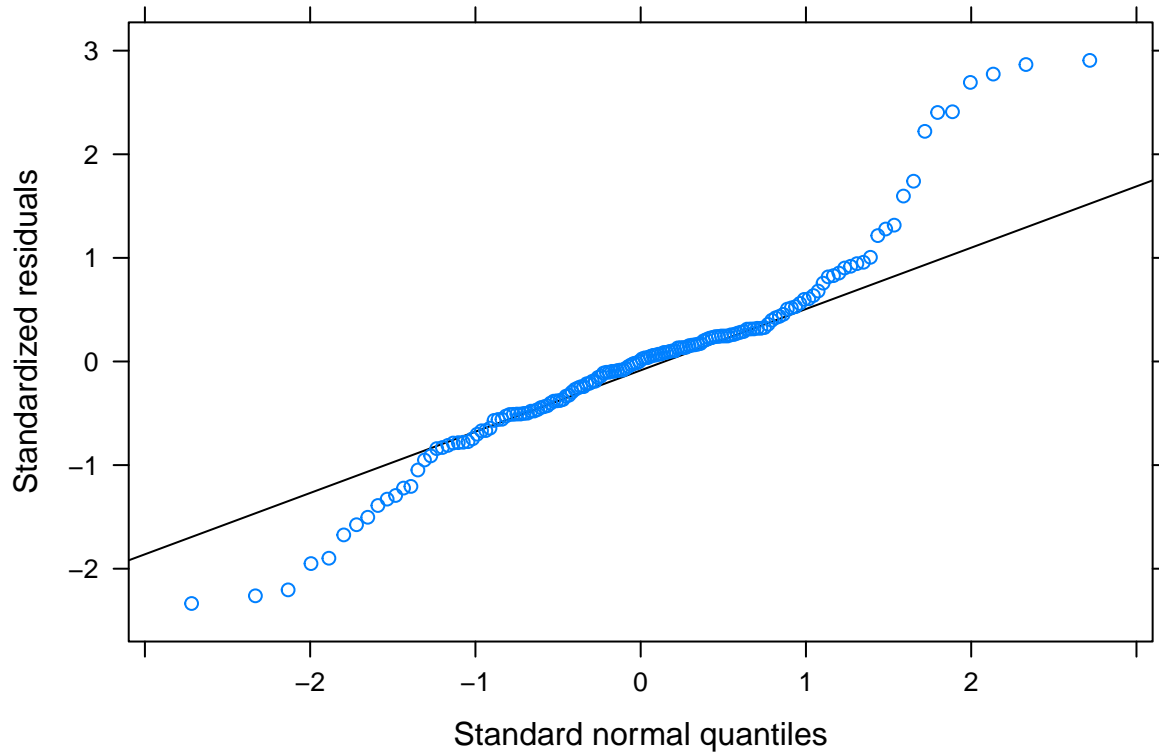
```
head(SumStats_phase)
```

```
## # A tibble: 6 x 9
## # Groups:   Genotype, Transmitter, Sleep.Stage [3]
##   Genotype Transmitter Sleep.Stage phase daily_bouts daily_duration min_bout
##   <fct>     <fct>      <fct> <fct>         <dbl>      <dbl>      <int>
## 1 McKO      1050240      P          dark           66.7       1583.       10
## 2 McKO      1050240      P          light          117.       3420.       10
## 3 McKO      1050240      S          dark           90.3      15800.       10
## 4 McKO      1050240      S          light          169      29763.       10
## 5 McKO      1050240      W          dark           108.      25880.       10
## 6 McKO      1050240      W          light          138       9907.       10
## # ... with 2 more variables: mean_bout <dbl>, max_bout <int>
```

```
lm_n_bouts <- lmer(daily_bouts ~ Sleep.Stage * phase * Genotype + (1 | Transmitter), data=SumStats_phase)
plot(lm_n_bouts, type=c("p","smooth"), col.line=1)
```



```
lattice::qqmath(lm_n_bouts)
```



```
anova(lm_n_bouts)
```

```
## Analysis of Variance Table
```

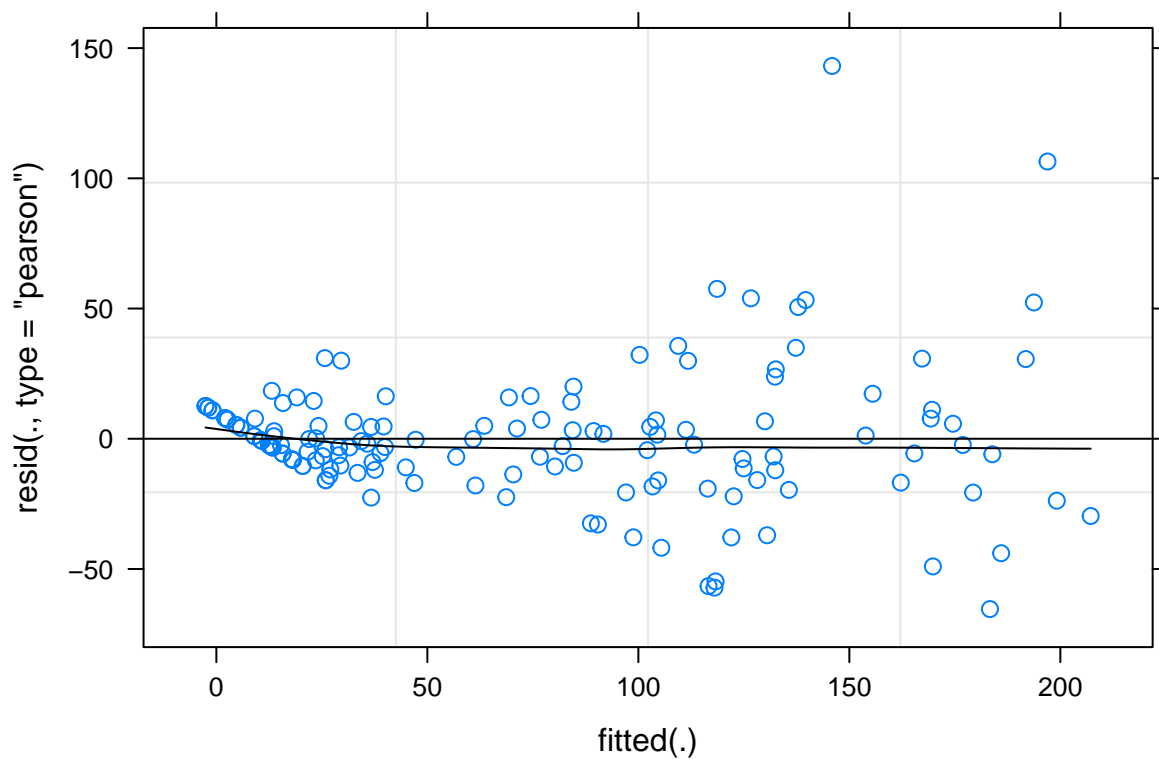
##		npair	Sum Sq	Mean Sq	F value
##	Sleep.Stage	3	813578	271193	146.4365
##	phase	1	25066	25066	13.5350
##	Genotype	1	176	176	0.0948
##	Sleep.Stage:phase	3	38793	12931	6.9824
##	Sleep.Stage:Genotype	3	634	211	0.1142
##	phase:Genotype	1	354	354	0.1913
##	Sleep.Stage:phase:Genotype	3	304	101	0.0547

```
emmeans(lm_n_bouts, pairwise ~ Genotype | Sleep.Stage * phase)$contrasts
```

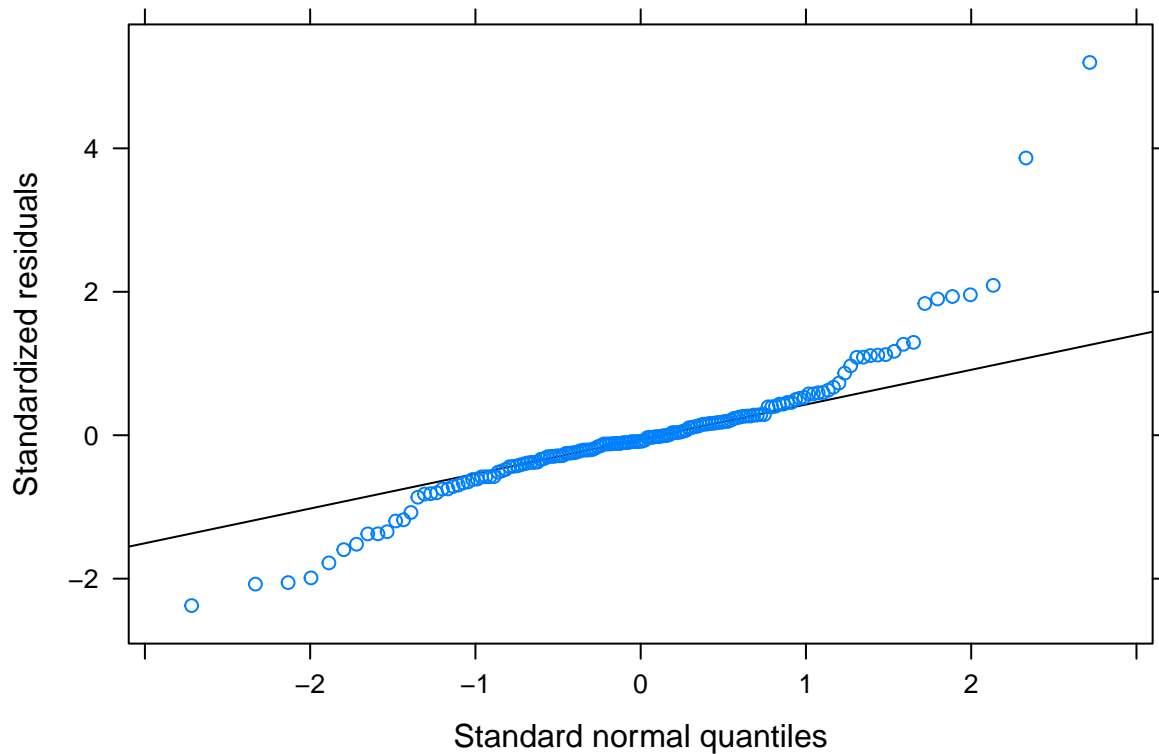
```
## Sleep.Stage = P, phase = dark:
## contrast estimate SE df t.ratio p.value
## McKO - WT -4.46 21.9 109 -0.203 0.8395
##
## Sleep.Stage = S, phase = dark:
## contrast estimate SE df t.ratio p.value
## McKO - WT -5.49 21.9 109 -0.250 0.8030
##
## Sleep.Stage = W, phase = dark:
## contrast estimate SE df t.ratio p.value
## McKO - WT -10.43 21.9 109 -0.475 0.6356
```

```
##
## Sleep.Stage = X, phase = dark:
## contrast estimate SE df t.ratio p.value
## McKO - WT -6.40 21.9 109 -0.292 0.7711
##
## Sleep.Stage = P, phase = light:
## contrast estimate SE df t.ratio p.value
## McKO - WT 10.93 21.9 109 0.498 0.6195
##
## Sleep.Stage = S, phase = light:
## contrast estimate SE df t.ratio p.value
## McKO - WT -4.96 21.9 109 -0.226 0.8215
##
## Sleep.Stage = W, phase = light:
## contrast estimate SE df t.ratio p.value
## McKO - WT -4.68 21.9 109 -0.213 0.8316
##
## Sleep.Stage = X, phase = light:
## contrast estimate SE df t.ratio p.value
## McKO - WT -3.60 21.9 109 -0.164 0.8699
##
## Degrees-of-freedom method: kenward-roger
```

```
lm_mean_length <- lmer(mean_bout ~ Sleep.Stage * phase * Genotype + (1 | Transmitter), data=SumStats_ph
plot(lm_mean_length, type=c("p","smooth"), col.line=1)
```



```
lattice::qqmath(lm_mean_length)
```



```
anova(lm_mean_length)
```

```
## Analysis of Variance Table
##               npar Sum Sq Mean Sq  F value
## Sleep.Stage      3 437815   145938  192.4372
## phase            1   1922     1922    2.5345
## Genotype          1     35       35    0.0462
## Sleep.Stage:phase  3   76820   25607   33.7653
## Sleep.Stage:Genotype 3    3306    1102    1.4533
## phase:Genotype     1        1        1    0.0018
## Sleep.Stage:phase:Genotype 3     978     326    0.4298
```

```
emmeans(lm_mean_length, pairwise ~ Genotype | Sleep.Stage * phase)$contrasts
```

```
## Sleep.Stage = P, phase = dark:
## contrast estimate SE df t.ratio p.value
## McKO - WT   -5.146 13.6 120  -0.378  0.7062
##
## Sleep.Stage = S, phase = dark:
## contrast estimate SE df t.ratio p.value
## McKO - WT    9.419 13.6 120   0.692  0.4905
##
```

```
## Sleep.Stage = W, phase = dark:
## contrast estimate SE df t.ratio p.value
## McKO - WT 0.767 13.6 120 0.056 0.9552
##
## Sleep.Stage = X, phase = dark:
## contrast estimate SE df t.ratio p.value
## McKO - WT 0.000 13.6 120 0.000 1.0000
##
## Sleep.Stage = P, phase = light:
## contrast estimate SE df t.ratio p.value
## McKO - WT -5.784 13.6 120 -0.425 0.6718
##
## Sleep.Stage = S, phase = light:
## contrast estimate SE df t.ratio p.value
## McKO - WT 24.825 13.6 120 1.823 0.0708
##
## Sleep.Stage = W, phase = light:
## contrast estimate SE df t.ratio p.value
## McKO - WT -12.489 13.6 120 -0.917 0.3610
##
## Sleep.Stage = X, phase = light:
## contrast estimate SE df t.ratio p.value
## McKO - WT 0.000 13.6 120 0.000 1.0000
##
## Degrees-of-freedom method: kenward-roger
```

```
lm_max_length <- lmer(max_bout ~ Sleep.Stage * phase * Genotype + (1 | Transmitter), data=SumStats_phase)
#plot(lm_max_length, type=c("p", "smooth"), col.line=1)
#lattice::qqmath(lm_max_length)
anova(lm_max_length)
```

```
## Analysis of Variance Table
##
##          npar    Sum Sq Mean Sq F value
## Sleep.Stage      3 88814079 29604693 63.6687
## phase            1  843042   843042  1.8131
## Genotype         1  170326   170326  0.3663
## Sleep.Stage:phase  3  7416195 2472065  5.3165
## Sleep.Stage:Genotype  3  244168    81389  0.1750
## phase:Genotype     1  192675   192675  0.4144
## Sleep.Stage:phase:Genotype  3  313821   104607  0.2250
```

```
emmeans(lm_max_length, pairwise ~ Genotype | Sleep.Stage * phase)$contrasts
```

```
## Sleep.Stage = P, phase = dark:
## contrast estimate SE df t.ratio p.value
## McKO - WT -18.78 322 133 -0.058 0.9536
##
## Sleep.Stage = S, phase = dark:
## contrast estimate SE df t.ratio p.value
## McKO - WT -425.78 322 133 -1.323 0.1881
##
## Sleep.Stage = W, phase = dark:
## contrast estimate SE df t.ratio p.value
```



```
## McKO - WT -162.78 322 133 -0.506 0.6139
##
## Sleep.Stage = X, phase = dark:
## contrast estimate SE df t.ratio p.value
## McKO - WT 0.00 322 133 0.000 1.0000
##
## Sleep.Stage = P, phase = light:
## contrast estimate SE df t.ratio p.value
## McKO - WT -46.56 322 133 -0.145 0.8852
##
## Sleep.Stage = S, phase = light:
## contrast estimate SE df t.ratio p.value
## McKO - WT 4.00 322 133 0.012 0.9901
##
## Sleep.Stage = W, phase = light:
## contrast estimate SE df t.ratio p.value
## McKO - WT 5.67 322 133 0.018 0.9860
##
## Sleep.Stage = X, phase = light:
## contrast estimate SE df t.ratio p.value
## McKO - WT 0.00 322 133 0.000 1.0000
##
## Degrees-of-freedom method: kenward-roger
```

```
lm_duration <- lmer(daily_duration ~ Sleep.Stage * phase * Genotype + (1 | Transmitter), data=SumStats_
```

```
## boundary (singular) fit: see ?isSingular
```

```
#plot(lm_duration, type=c("p", "smooth"), col.line=1)
#lattice::qqmath(lm_duration)
anova(lm_duration)
```

```
## Analysis of Variance Table
##               npar      Sum Sq    Mean Sq    F value
## Sleep.Stage      3 1.4569e+10 4856432337 1443.3593
## phase            1 3.5464e+05    354638    0.1054
## Genotype         1 1.5500e+02      155    0.0000
## Sleep.Stage:phase 3 3.0043e+09 1001440234 297.6337
## Sleep.Stage:Genotype 3 3.7574e+07 12524544    3.7224
## phase:Genotype    1 3.6430e+03     3643    0.0011
## Sleep.Stage:phase:Genotype 3 5.7953e+06 1931773    0.5741
```

```
emmeans(lm_duration, pairwise ~ Genotype | Sleep.Stage * phase)$contrasts
```

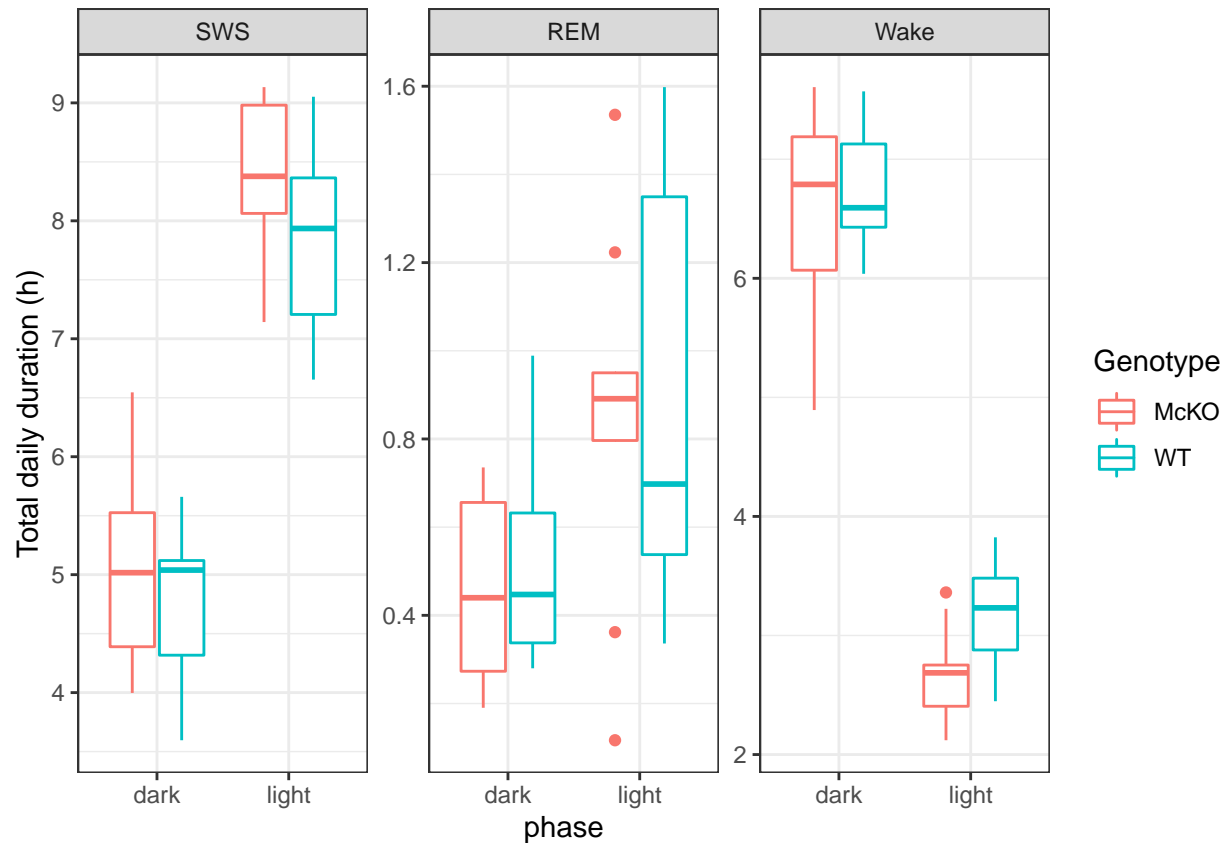
```
## Sleep.Stage = P, phase = dark:
## contrast estimate SE df t.ratio p.value
## McKO - WT -245 843 136 -0.290 0.7719
##
## Sleep.Stage = S, phase = dark:
## contrast estimate SE df t.ratio p.value
## McKO - WT 1023 843 136 1.214 0.2268
##
```

```
## Sleep.Stage = W, phase = dark:
## contrast estimate SE df t.ratio p.value
## McKO - WT      -667 843 136 -0.792 0.4300
##
## Sleep.Stage = X, phase = dark:
## contrast estimate SE df t.ratio p.value
## McKO - WT      -64 843 136 -0.076 0.9396
##
## Sleep.Stage = P, phase = light:
## contrast estimate SE df t.ratio p.value
## McKO - WT      -149 843 136 -0.177 0.8596
##
## Sleep.Stage = S, phase = light:
## contrast estimate SE df t.ratio p.value
## McKO - WT      2022 843 136  2.399 0.0178
##
## Sleep.Stage = W, phase = light:
## contrast estimate SE df t.ratio p.value
## McKO - WT      -1868 843 136 -2.216 0.0284
##
## Sleep.Stage = X, phase = light:
## contrast estimate SE df t.ratio p.value
## McKO - WT      -36 843 136 -0.043 0.9660
##
## Degrees-of-freedom method: kenward-roger
```

```
SumStats_phase$Sleep.Stage <- recode_factor(SumStats_phase$Sleep.Stage, S = "SWS", P = "REM", X = "Artifact")
```

```
daily_duration_plot <- SumStats_phase %>% filter(Sleep.Stage != "Artifact") %>%
  ggplot(aes(x=phase, y=(daily_duration/3600))) +
  facet_wrap(~Sleep.Stage, scales="free_y") +
  geom_boxplot(aes(color=Genotype)) +
  ylab("Total daily duration (h)") +
  theme_bw()
```

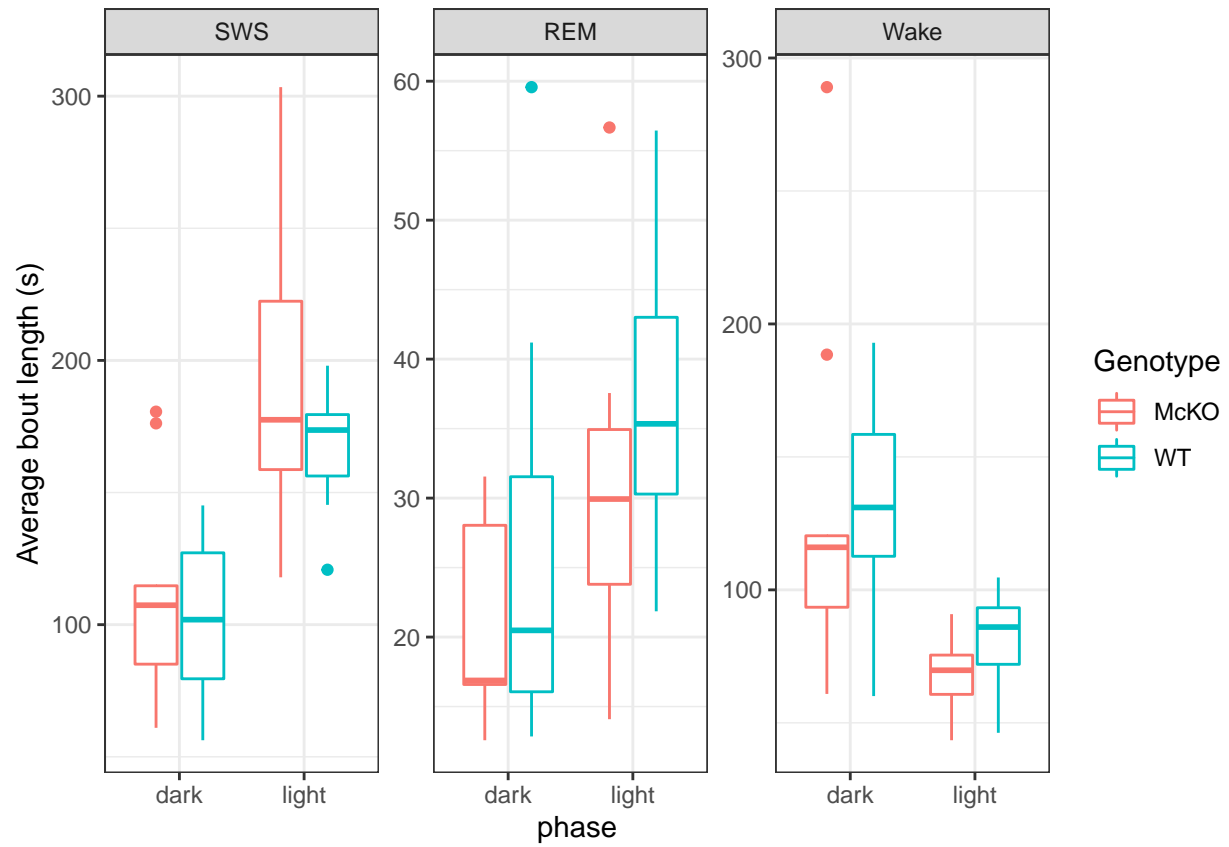
```
daily_duration_plot
```



```
#ggsave(filename="../figures/daily_duration_plot.svg", plot=daily_duration_plot, height=4, width=7)
```

```
length_phase_plot <- SumStats_phase %>% filter(Sleep.Stage != "Artifact") %>%
  ggplot(aes(x=phase, y=mean_bout)) +
  facet_wrap(~Sleep.Stage, scales="free_y") +
  geom_boxplot(aes(color=Genotype)) +
  ylab("Average bout length (s)") +
  theme_bw()
```

```
length_phase_plot
```



```
#ggsave(filename="../figures/length_phase_plot.svg", plot=length_phase_plot, height=4, width=7)
```