

# 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) %>%
  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'. You can override using
head(SumStats_daily)
```

```
## # A tibble: 6 x 10
## # Groups:   Genotype, Transmitter, Sleep.Stage [2]
```

```
##   Genotype Transmitter Sleep.Stage   Day n_bouts total_duration min_bout_length
##   <fct>    <fct>      <fct>      <int>  <int>          <int>          <int>
## 1 McKO     1050240     P           1    138           4760             10
## 2 McKO     1050240     P           2    183           5340             10
## 3 McKO     1050240     P           3    229           4910             10
## 4 McKO     1050240     S           1    237          45220             10
## 5 McKO     1050240     S           2    257          45180             10
## 6 McKO     1050240     S           3    284          46290             10
## # ... with 3 more variables: mean_bout_length <dbl>, max_bout_length <int>,
## #   sd_bout_length <dbl>
```

```
SumStats_daily_n <- SumStats_daily %>% group_by(Genotype, Transmitter, Sleep.Stage) %>%
  summarise(mean_n_bouts_day = mean(n_bouts))
```

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

```
head(SumStats_daily_n)
```

```
## # A tibble: 6 x 4
## # Groups:   Genotype, Transmitter [2]
##   Genotype Transmitter Sleep.Stage mean_n_bouts_day
##   <fct>    <fct>      <fct>          <dbl>
## 1 McKO     1050240     P              183.
## 2 McKO     1050240     S              259.
## 3 McKO     1050240     W              246.
## 4 McKO     1050240     X               6
## 5 McKO     1050241     P             283.
## 6 McKO     1050241     S             234
```

```
SumStats_avg <- bouts %>% group_by(Genotype, Transmitter, Sleep.Stage) %>%
  summarise(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'. You can override using the `.groups` argument

```
head(SumStats_avg)
```

```
## # A tibble: 6 x 7
## # Groups:   Genotype, Transmitter [2]
##   Genotype Transmitter Sleep.Stage min_bout_length mean_bout_length
##   <fct>    <fct>      <fct>          <int>          <dbl>
## 1 McKO     1050240     P              10             27.3
## 2 McKO     1050240     S              10             176.
## 3 McKO     1050240     W              10             145.
## 4 McKO     1050240     X              10              10
## 5 McKO     1050241     P              10             28.6
## 6 McKO     1050241     S              10             171.
## # ... with 2 more variables: max_bout_length <int>, sd_bout_length <dbl>
```

```
SumStats_phase <- bouts %>% group_by(Genotype, Transmitter, Sleep.Stage, 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'. You can override using
head(SumStats_phase)

## # A tibble: 6 x 10
## # Groups:   Genotype, Transmitter, Sleep.Stage [3]
##   Genotype Transmitter Sleep.Stage phase n_bouts total_duration min_bout_length
##   <fct>     <fct>       <fct>   <fct>   <int>         <int>         <int>
## 1 McKO     1050240      P      dark     200          4750           10
## 2 McKO     1050240      P      light    350         10260           10
## 3 McKO     1050240      S      dark     271         47400           10
## 4 McKO     1050240      S      light    507         89290           10
## 5 McKO     1050240      W      dark     325         77640           10
## 6 McKO     1050240      W      light    414         29720           10
## # ... with 3 more variables: mean_bout_length <dbl>, max_bout_length <int>,
## #   sd_bout_length <dbl>

SumStats_phase_n <- SumStats_phase %>% group_by(Genotype, Transmitter, Sleep.Stage, phase) %>%
  summarise(mean_n_bouts_day = mean(n_bouts))

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

## # A tibble: 6 x 5
## # Groups:   Genotype, Transmitter, Sleep.Stage [3]
##   Genotype Transmitter Sleep.Stage phase mean_n_bouts_day
##   <fct>     <fct>       <fct>   <fct>         <dbl>
## 1 McKO     1050240      P      dark           200
## 2 McKO     1050240      P      light          350
## 3 McKO     1050240      S      dark           271
## 4 McKO     1050240      S      light          507
## 5 McKO     1050240      W      dark           325
## 6 McKO     1050240      W      light          414

SumStats_avg %>% group_by(Genotype, Sleep.Stage) %>%
  summarise(n = n(),
            mean_bout_length = mean(mean_bout_length),
            min_bout_length = min(min_bout_length),
            max_bout_length = max(max_bout_length))

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

## # A tibble: 8 x 6
## # Groups:   Genotype [2]
##   Genotype Sleep.Stage      n mean_bout_length min_bout_length max_bout_length
##   <fct>     <fct>    <int>         <dbl>         <int>         <int>
## 1 McKO     P          9          26.8           10           250
## 2 McKO     S          9         142.           10          3920
## 3 McKO     W          9          95.2           10          6310
## 4 McKO     X          9          10            10           10
## 5 WT       P         10          30.9           10           350
## 6 WT       S         10         127.           10          4390
## 7 WT       W         10         100.           10          5230
## 8 WT       X         10          10            10           10

```

```
SumStats_phase_grps <- SumStats_phase %>% group_by(Genotype, Sleep.Stage, phase) %>%
  summarise(n = n(),
            mean_bout_length = mean(mean_bout_length),
            min_bout_length = min(min_bout_length),
            max_bout_length = max(max_bout_length))
```

```
## `summarise()` has grouped output by 'Genotype', 'Sleep.Stage'. You can override using the `.groups` argument
head(SumStats_phase_grps)
```

```
## # A tibble: 6 x 7
## # Groups:   Genotype, Sleep.Stage [3]
##   Genotype Sleep.Stage phase      n mean_bout_length min_bout_length
##   <fct>    <fct>    <fct> <int>          <dbl>          <int>
## 1 McKO     P        dark     9          20.5            10
## 2 McKO     P        light    9          31.0            10
## 3 McKO     S        dark     9         107.            10
## 4 McKO     S        light    9         190.            10
## 5 McKO     W        dark     9         120.            10
## 6 McKO     W        light    9         67.9            10
## # ... with 1 more variable: max_bout_length <int>
```

```
SumStats_phase_n_grps <- SumStats_phase_n %>% group_by(Genotype, Sleep.Stage, phase) %>%
  summarise(n = n(),
            mean_n_bouts_day = mean(mean_n_bouts_day))
```

```
## `summarise()` has grouped output by 'Genotype', 'Sleep.Stage'. You can override using the `.groups` argument
head(SumStats_phase_n_grps)
```

```
## # A tibble: 6 x 5
## # Groups:   Genotype, Sleep.Stage [3]
##   Genotype Sleep.Stage phase      n mean_n_bouts_day
##   <fct>    <fct>    <fct> <int>          <dbl>
## 1 McKO     P        dark     9          378.
## 2 McKO     P        light    9          418.
## 3 McKO     S        dark     9          881.
## 4 McKO     S        light    9          779.
## 5 McKO     W        dark     9         1005.
## 6 McKO     W        light    9          673.
```

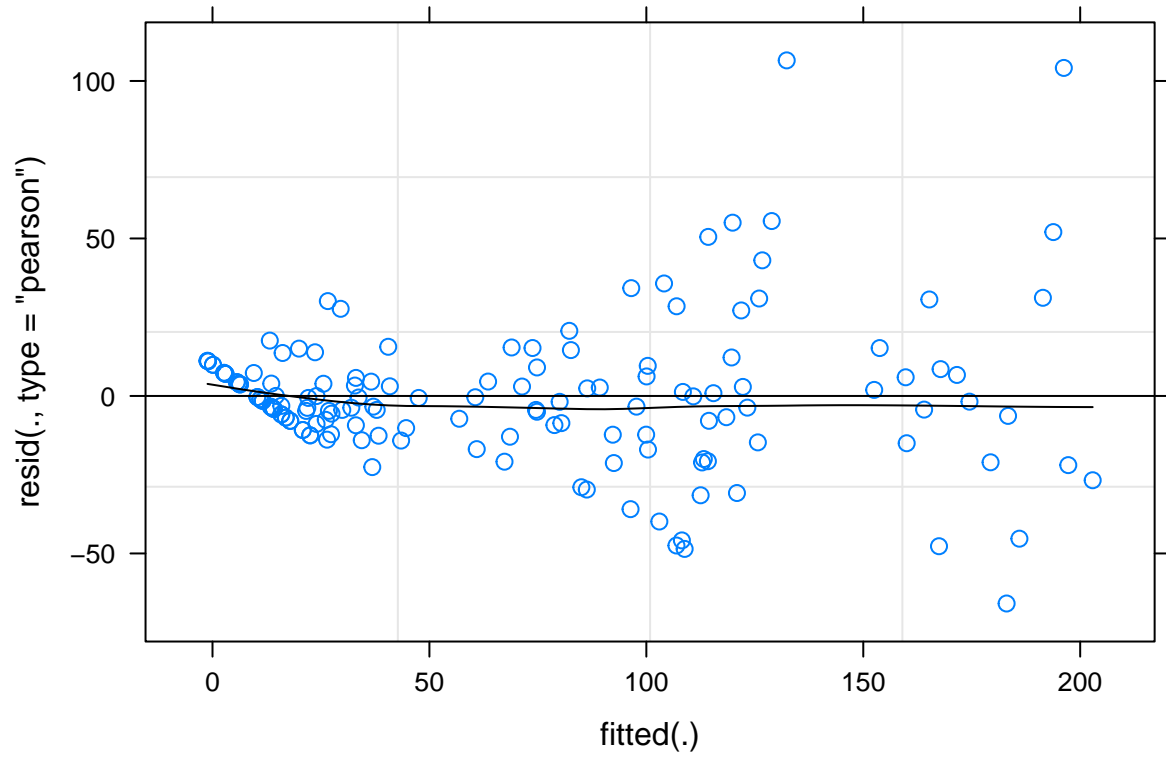
```
lm_n_bouts <- lmer(mean_n_bouts_day ~ Sleep.Stage * phase * Genotype + (1 | Transmitter), data=SumStats_phase_n_grps)
#plot(lm_n_bouts, type=c("p", "smooth"), col.line=1)
#lattice::qqmath(lm_n_bouts)
anova(lm_n_bouts)
```

```
## Analysis of Variance Table
##               npar    Sum Sq Mean Sq  F value
## Sleep.Stage      3 16786640 5595547 109.8216
## phase            1   453224  453224   8.8952
## Genotype          1     566     566   0.0111
## Sleep.Stage:phase  3   716742  238914   4.6891
## Sleep.Stage:Genotype 3     3057   1019   0.0200
## phase:Genotype     1     1379   1379   0.0271
## Sleep.Stage:phase:Genotype 3     5461   1820   0.0357
```

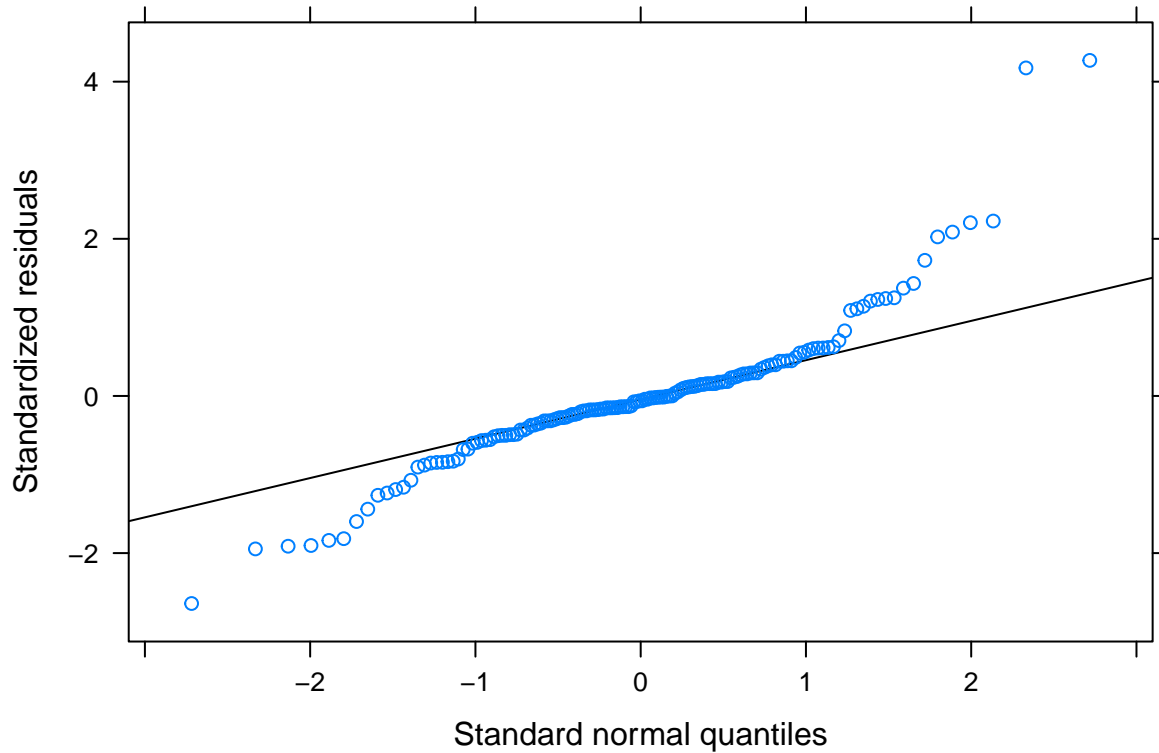
```
emmeans(lm_n_bouts, pairwise ~ Genotype | Sleep.Stage * phase)$contrasts
```

```
## Sleep.Stage = P, phase = dark:
## contrast estimate SE df t.ratio p.value
## McKO - WT -23.84 141 54.8 -0.169 0.8665
##
## Sleep.Stage = S, phase = dark:
## contrast estimate SE df t.ratio p.value
## McKO - WT 8.97 141 54.8 0.064 0.9496
##
## Sleep.Stage = W, phase = dark:
## contrast estimate SE df t.ratio p.value
## McKO - WT -28.18 141 54.8 -0.200 0.8425
##
## Sleep.Stage = X, phase = dark:
## contrast estimate SE df t.ratio p.value
## McKO - WT -24.29 141 54.8 -0.172 0.8640
##
## Sleep.Stage = P, phase = light:
## contrast estimate SE df t.ratio p.value
## McKO - WT 20.09 141 54.8 0.142 0.8873
##
## Sleep.Stage = S, phase = light:
## contrast estimate SE df t.ratio p.value
## McKO - WT -14.33 141 54.8 -0.102 0.9195
##
## Sleep.Stage = W, phase = light:
## contrast estimate SE df t.ratio p.value
## McKO - WT -18.44 141 54.8 -0.131 0.8965
##
## Sleep.Stage = X, phase = light:
## contrast estimate SE df t.ratio p.value
## McKO - WT -6.40 141 54.8 -0.045 0.9640
##
## Degrees-of-freedom method: kenward-roger
```

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



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



```
anova(lm_mean_length)
```

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

	npair	Sum Sq	Mean Sq	F value
## Sleep.Stage	3	407882	135961	218.4922
## phase	1	3817	3817	6.1332
## Genotype	1	85	85	0.1372
## Sleep.Stage:phase	3	70113	23371	37.5577
## Sleep.Stage:Genotype	3	3678	1226	1.9700
## phase:Genotype	1	4	4	0.0065
## Sleep.Stage:phase:Genotype	3	923	308	0.4945

```
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 -5.21 12.3 120 -0.422 0.6739
##
## Sleep.Stage = S, phase = dark:
## contrast estimate SE df t.ratio p.value
## McKO - WT 11.22 12.3 120 0.908 0.3655
##
## Sleep.Stage = W, phase = dark:
## contrast estimate SE df t.ratio p.value
## McKO - WT 1.76 12.3 120 0.143 0.8869
##
## Sleep.Stage = X, phase = dark:
```

```

## contrast estimate SE df t.ratio p.value
## McKO - WT 0.00 12.3 120 0.000 1.0000
##
## Sleep.Stage = P, phase = light:
## contrast estimate SE df t.ratio p.value
## McKO - WT -5.76 12.3 120 -0.466 0.6420
##
## Sleep.Stage = S, phase = light:
## contrast estimate SE df t.ratio p.value
## McKO - WT 26.70 12.3 120 2.162 0.0326
##
## Sleep.Stage = W, phase = light:
## contrast estimate SE df t.ratio p.value
## McKO - WT -10.55 12.3 120 -0.854 0.3947
##
## Sleep.Stage = X, phase = light:
## contrast estimate SE df t.ratio p.value
## McKO - WT 0.00 12.3 120 0.000 1.0000
##
## Degrees-of-freedom method: kenward-roger

lm_max_length <- lmer(max_bout_length ~ Sleep.Stage * phase * Genotype + (1 | Transmitter), data=SumSta
#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
```

```
SumStats_phase %>% filter(Sleep.Stage == "S" & phase == "light") %>%
  ggplot(aes(x=Genotype, y=mean_bout_length)) +
  stat_summary(fun = 'mean', geom="bar") +
  stat_summary(fun.data = mean_se, geom = "errorbar", width=.2) +
  geom_point()
```

