Stroke Therapy

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```
##rm(list=ls())
set.seed(10302020)
library(mgcv)
```

```
## Loading required package: nlme
```

```
## This is mgcv 1.8-31. For overview type 'help("mgcv-package")'.
```

```
library(lme4)
```

```
## Loading required package: Matrix
```

```
##
## Attaching package: 'lme4'
```

```
## The following object is masked from 'package:nlme':
##
##     lmList
```

```
library(effects)
```

```
## Loading required package: carData
```

```
## lattice theme set by effectsTheme()
## See ?effectsTheme for details.
```

```
library(sjPlot)
```

```
## #refugeeswelcome
```

```
library(glmmTMB)
library(tidyverse)
```

```
## -- Attaching packages ---------------------------------------------- tidyverse 1.3.0 --
```

```
## v ggplot2 3.3.2     v purrr   0.3.4
## v tibble  3.0.3     v dplyr   1.0.2
## v tidyr   1.1.2     v stringr 1.4.0
## v readr   1.3.1     v forcats 0.5.0
```

```
## -- Conflicts ------------------------------------------------- tidyverse_conflicts() --
## x dplyr::collapse() masks nlme::collapse()
## x tidyr::expand()   masks Matrix::expand()
## x dplyr::filter()   masks stats::filter()
## x dplyr::lag()      masks stats::lag()
## x tidyr::pack()     masks Matrix::pack()
## x tidyr::unpack()   masks Matrix::unpack()
```

```
library(ggpubr)
```

```
## Registered S3 methods overwritten by 'car':
##   method                          from
##   influence.merMod                lme4
##   cooks.distance.influence.merMod lme4
##   dfbeta.influence.merMod         lme4
##   dfbetas.influence.merMod        lme4
```

```
library(rstatix)
```

```
##
## Attaching package: 'rstatix'
```

```
## The following object is masked from 'package:stats':
##
##     filter
```

```
## [1] 12960     7
```

```
##   amplitude replicate time montage paired.pulse lesional subjectID
## 1 0.7048035         1    1       1            1        1         1
## 2 0.4856873         2    1       1            1        1         1
## 3 0.5845642         3    1       1            1        1         1
## 4 0.5323792         4    1       1            1        1         1
## 5 0.6295776         5    1       1            1        1         1
## 6 0.5810547         6    1       1            1        1         1
```

```
## [1] 12960     7
```

```
##     pct.change replicate time montage paired.pulse lesional subjectID
## 1  21.39689214         1    1       1            1        1         1
## 2 -16.34416374         2    1       1            1        1         1
## 3   0.68661914         3    1       1            1        1         1
## 4  -8.30184960         4    1       1            1        1         1
## 5   8.43983048         5    1       1            1        1         1
## 6   0.08213148         6    1       1            1        1         1
```

```
table(unlist(x[,-1]==x2[,-1]))
```

```
##
##  TRUE
## 77760
```

```
x$pct.change<-x2$pct.change
rm(x2)
x$condBySubject<-paste0(x$subject,x$montage,x$paired.pulse,x$lesional)
x$replicate<-factor(x$replicate)
x$time<-factor(x$time,levels=c(1:5), labels=c("baseline","post1","post2","post3","post4"))
x$montage<-factor(x$montage,levels=c(1:3), labels=c("anodal","bihemi","cathodal"))
x$paired.pulse<-factor(x$paired.pulse,levels=c(1,2,3), labels=c("single","inhibitory","excitory"))
x$lesional<-factor(x$lesional,levels=c(1:2), labels=c("lesional","nonlesional"))
x$subjectID<-factor(x$subjectID)
head(x)
```

```
##   amplitude replicate     time montage paired.pulse lesional subjectID
## 1 0.7048035         1 baseline  anodal       single lesional         1
## 2 0.4856873         2 baseline  anodal       single lesional         1
## 3 0.5845642         3 baseline  anodal       single lesional         1
## 4 0.5323792         4 baseline  anodal       single lesional         1
## 5 0.6295776         5 baseline  anodal       single lesional         1
## 6 0.5810547         6 baseline  anodal       single lesional         1
##     pct.change condBySubject
## 1  21.39689214          1111
## 2 -16.34416374          1111
## 3   0.68661914          1111
## 4  -8.30184960          1111
## 5   8.43983048          1111
## 6   0.08213148          1111
```

```
bl<-x[x$time=="baseline",]
## baseline mean amplitude by condtion:
bl.means<-tapply(bl$amplitude,bl$condBySubject,mean)
summary(bl.means)
```

```
##    Min. 1st Qu.  Median    Mean 3rd Qu.    Max.    NA's
## 0.03237 0.43442 0.73307 0.85328 1.24316 2.66067       1
```

```
length(bl.means)
```

```
## [1] 324
```

```
length(unique(bl$condBySubject))
```

```
## [1] 324
```

```
x<-merge(x,bl.means,by.x="condBySubject",by.y=0,all.x=TRUE)
colnames(x)[colnames(x)=="y"]<-"BLmeanAmp"
head(x)
```

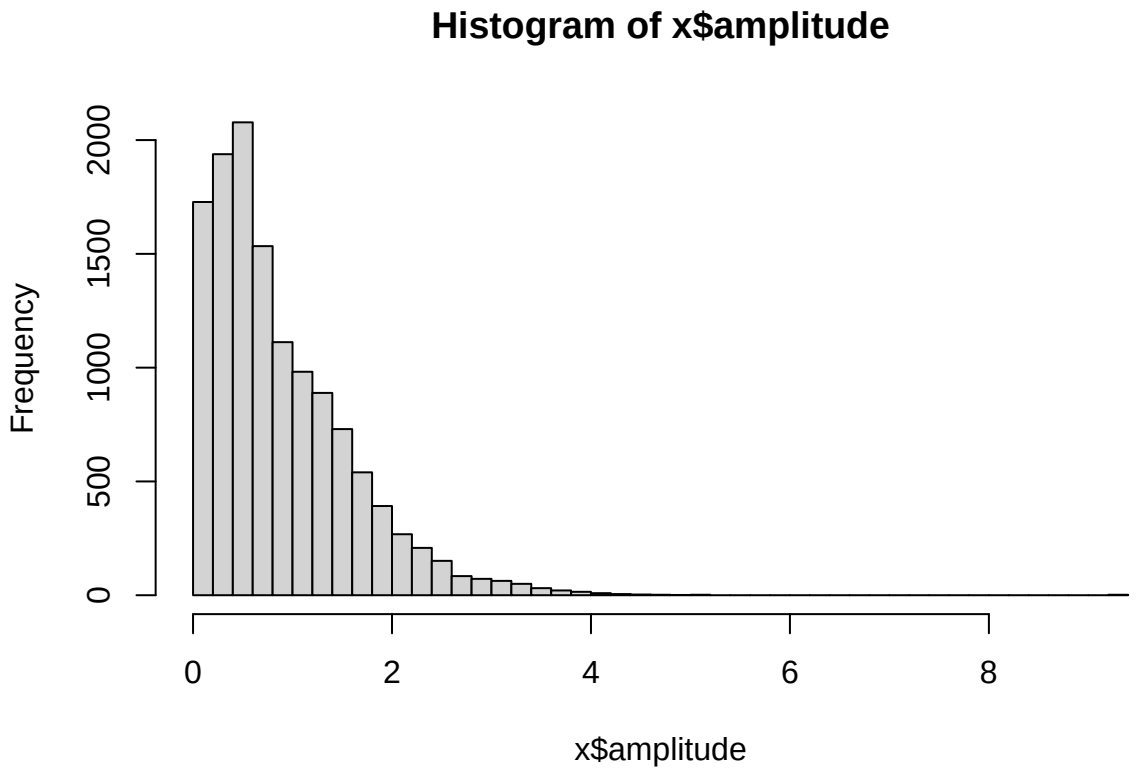
```
##   condBySubject amplitude replicate     time montage paired.pulse lesional
## 1         10111 0.4995728         1 baseline  anodal       single lesional
## 2         10111 0.5133057         2 baseline  anodal       single lesional
## 3         10111 0.3581238         3 baseline  anodal       single lesional
## 4         10111 0.6427002         4 baseline  anodal       single lesional
## 5         10111 0.5168152         5 baseline  anodal       single lesional
## 6         10111 0.6385803         6 baseline  anodal       single lesional
##   subjectID pct.change BLmeanAmp
## 1        10  -8.894222 0.5483437
```

```
## 2        10  -6.389787 0.5483437
## 3        10 -34.689902 0.5483437
## 4        10  17.207555 0.5483437
## 5        10  -5.749765 0.5483437
## 6        10  16.456225 0.5483437
```

```
summary(x$amplitude)
```

```
##    Min. 1st Qu.  Median    Mean 3rd Qu.    Max.    NA's
## 0.01755 0.35431 0.68832 0.88516 1.26598 9.31839      50
```

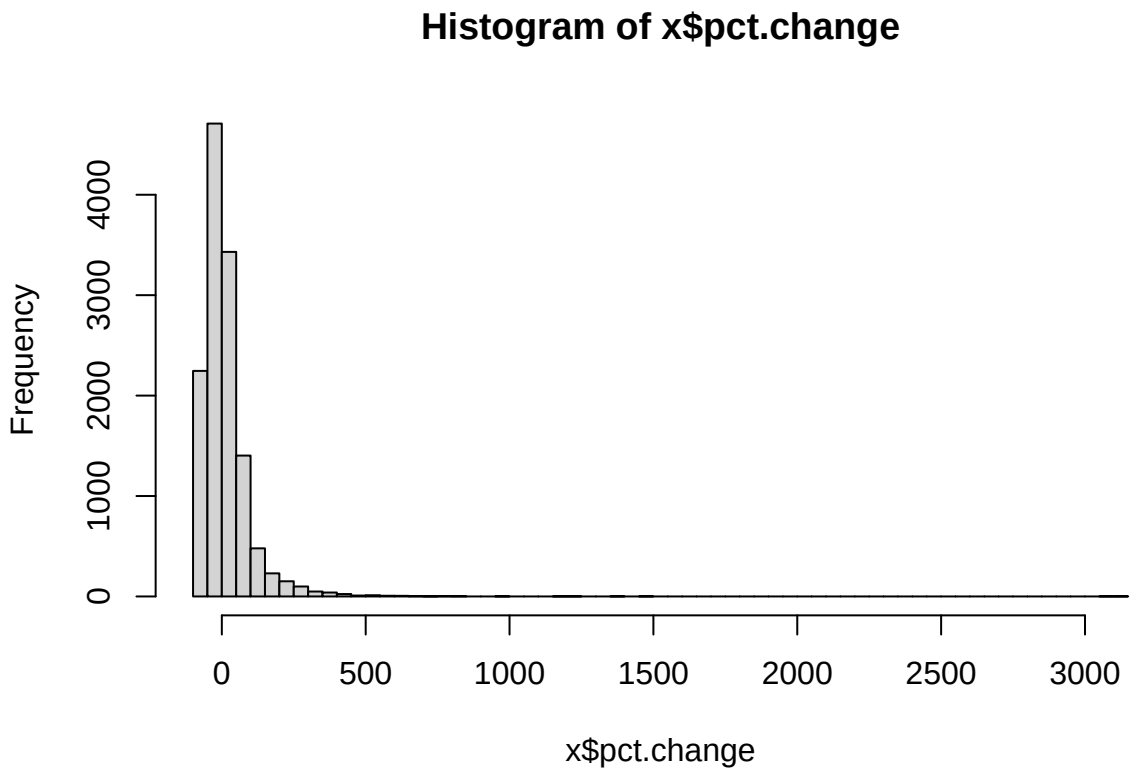
```
hist(x$amplitude,nclass=50)
```




```
summary(x$pct.change)
```

```
##    Min. 1st Qu.  Median    Mean 3rd Qu.    Max.    NA's
##  -99.17  -36.59   -4.48   11.18   35.51 3101.73      50
```

```
hist(x$pct.change,nclass=50)
```




```
table(x$replicate,useNA="always")
```

```
##
##    1    2    3    4    5    6    7    8 <NA>
## 1620 1620 1620 1620 1620 1620 1620 1620    0
```

```
table(x$time,useNA="always")
```

```
##
## baseline    post1    post2    post3    post4     <NA>
##     2592     2592     2592     2592     2592        0
```

```
table(x$montage,useNA="always")
```

```
##
##   anodal   bihemi cathodal     <NA>
##     4320     4320     4320        0
```

```
table(x$paired.pulse,useNA="always")
```

```
##
##     single inhibitory   excitory       <NA>
##       4320       4320       4320          0
```

```
table(x$lesional,useNA="always")
```

```
##
##    lesional nonlesional        <NA>
##        6480        6480           0
```

```r
table(x$subjectID,useNA="always")
```

```
##
##    1    2    3    4    5    6    7    8    9   10   11   12   13   14   15   16
##  720  720  720  720  720  720  720  720  720  720  720  720  720  720  720  720
##   17   18 <NA>
##  720  720    0
```

```r
x$amplitude <- scale(x$amplitude)
```

Linear Mixed Models

```r
library(lmerTest)
```

```
##
## Attaching package: 'lmerTest'

## The following object is masked from 'package:lme4':
##
##     lmer

## The following object is masked from 'package:stats':
##
##     step
```

```r
#mV = BLmV + Time*Montage + (Time|SubjectID)
lmm <- lmer(amplitude ~ BLmeanAmp + time*montage + (time|subjectID), data=x)
```

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

```r
summary(lmm)
```

```
## Linear mixed model fit by REML. t-tests use Satterthwaite's method [
## lmerModLmerTest]
## Formula: amplitude ~ BLmeanAmp + time * montage + (time | subjectID)
##    Data: x
##
## REML criterion at convergence: 26710.5
##
## Scaled residuals:
##     Min      1Q  Median      3Q     Max
## -4.9376 -0.5029 -0.1121  0.3678 17.9889
##
## Random effects:
##  Groups    Name        Variance Std.Dev. Corr
##  subjectID (Intercept) 0.002921 0.05405
##            timepost1   0.040813 0.20202  0.92
##            timepost2   0.036480 0.19100  0.11 0.43
##            timepost3   0.060996 0.24697  0.27 0.36 0.72
##            timepost4   0.018932 0.13759  0.39 0.48 0.66 0.93
##  Residual              0.458577 0.67718
## Number of obs: 12871, groups:  subjectID, 18
##
```

```
## Fixed effects:
##                              Estimate Std. Error         df t value Pr(>|t|)
## (Intercept)                -1.058e+00  2.839e-02  1.173e+02 -37.251  < 2e-16 ***
## BLmeanAmp                   1.183e+00  1.270e-02  6.474e+03  93.114  < 2e-16 ***
## timepost1                  -1.093e-02  5.770e-02  2.772e+01  -0.189  0.85119
## timepost2                   5.083e-02  5.557e-02  2.877e+01   0.915  0.36797
## timepost3                   1.104e-01  6.671e-02  2.413e+01   1.656  0.11076
## timepost4                  -9.396e-02  4.597e-02  3.943e+01  -2.044  0.04767 *
## montagebihemi               4.978e-03  3.258e-02  1.279e+04   0.153  0.87858
## montagecathodal             5.313e-03  3.266e-02  1.279e+04   0.163  0.87077
## timepost1:montagebihemi     1.264e-01  4.608e-02  1.279e+04   2.742  0.00611 **
## timepost2:montagebihemi     1.005e-02  4.608e-02  1.279e+04   0.218  0.82728
## timepost3:montagebihemi    -6.564e-02  4.608e-02  1.279e+04  -1.424  0.15436
## timepost4:montagebihemi     1.454e-01  4.608e-02  1.279e+04   3.155  0.00161 **
## timepost1:montagecathodal   1.802e-01  4.619e-02  1.279e+04   3.902 9.59e-05 ***
## timepost2:montagecathodal   3.673e-02  4.619e-02  1.279e+04   0.795  0.42647
## timepost3:montagecathodal  -1.597e-02  4.665e-02  1.280e+04  -0.342  0.73207
## timepost4:montagecathodal   1.140e-01  4.618e-02  1.279e+04   2.469  0.01356 *
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

##
## Correlation matrix not shown by default, as p = 16 > 12.
## Use print(x, correlation=TRUE)  or
##     vcov(x)        if you need it

## convergence code: 0
## boundary (singular) fit: see ?isSingular
```

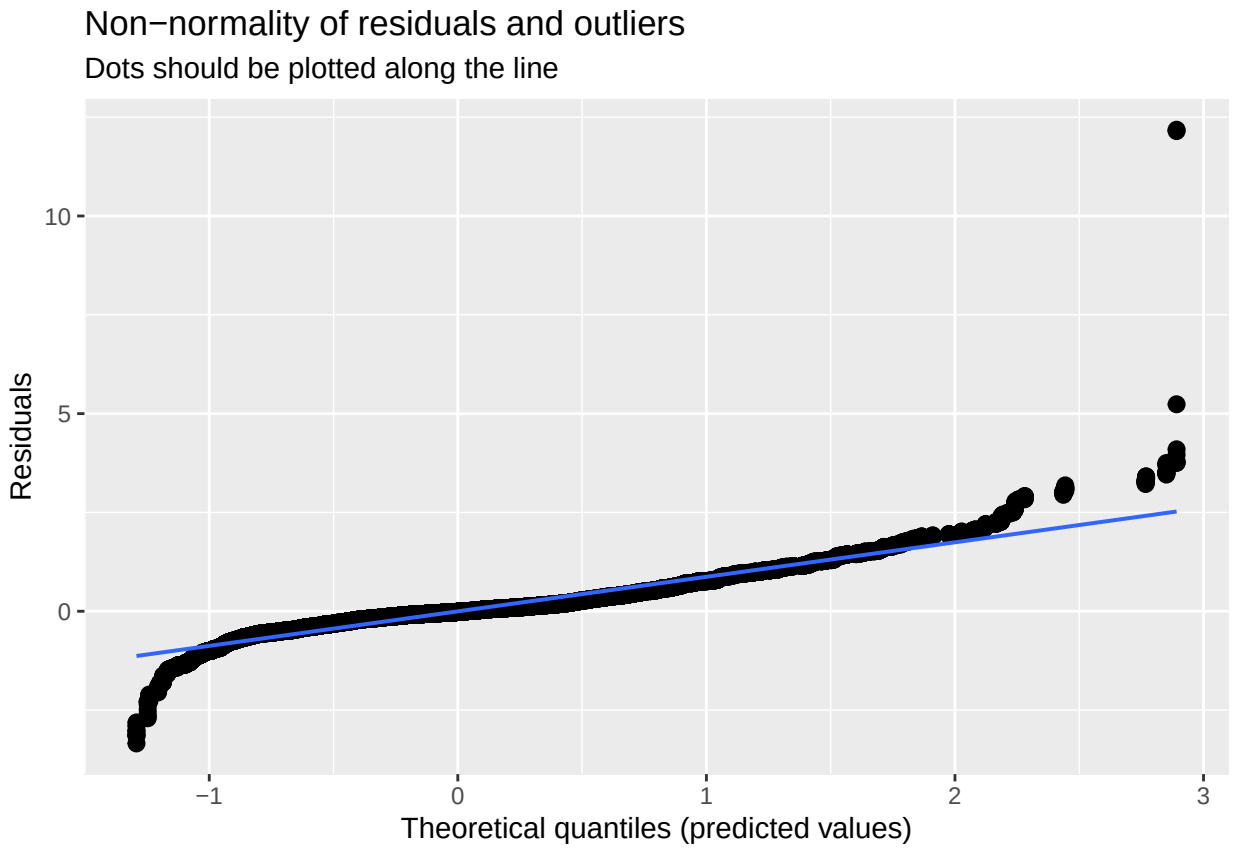
Based on the p-values, baseline mV and montage are shown to be significant. Some interactions between levels of time and montage are also significant, but none of the main effects of time is significant.

Check LMM Assumptions

```
#Try with model_plot (argument for type can be varied)
plot_model(lmm, type='diag')
```

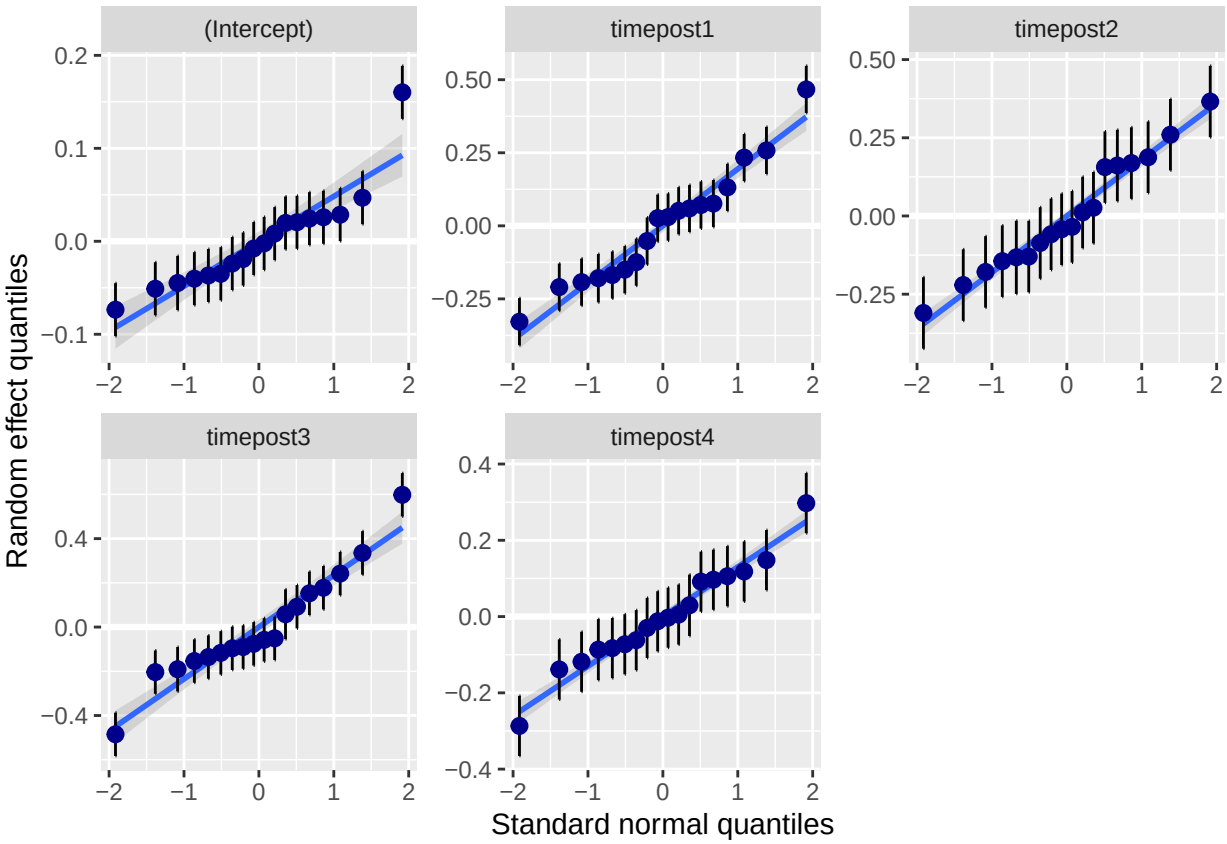
```
## [[1]]

## `geom_smooth()` using formula 'y ~ x'
```

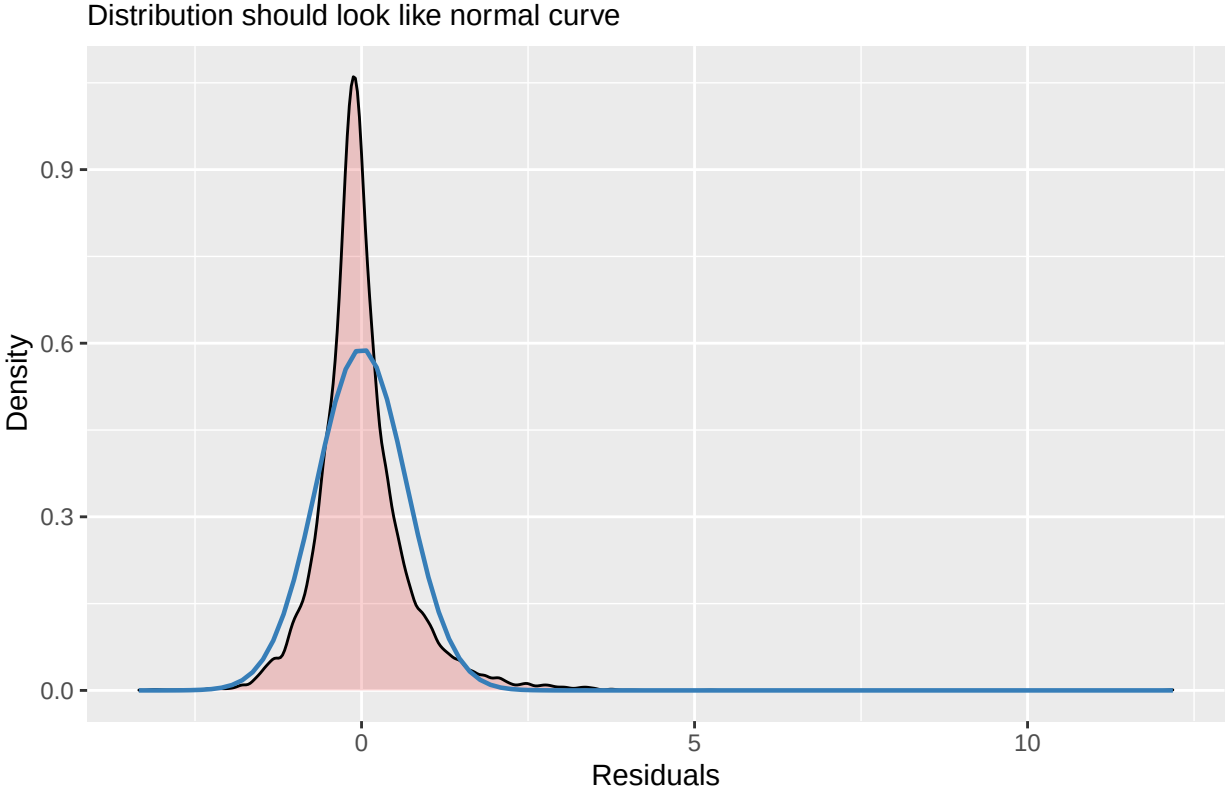



```
##
## [[2]]
## [[2]]$subjectID

## `geom_smooth()` using formula 'y ~ x'
```

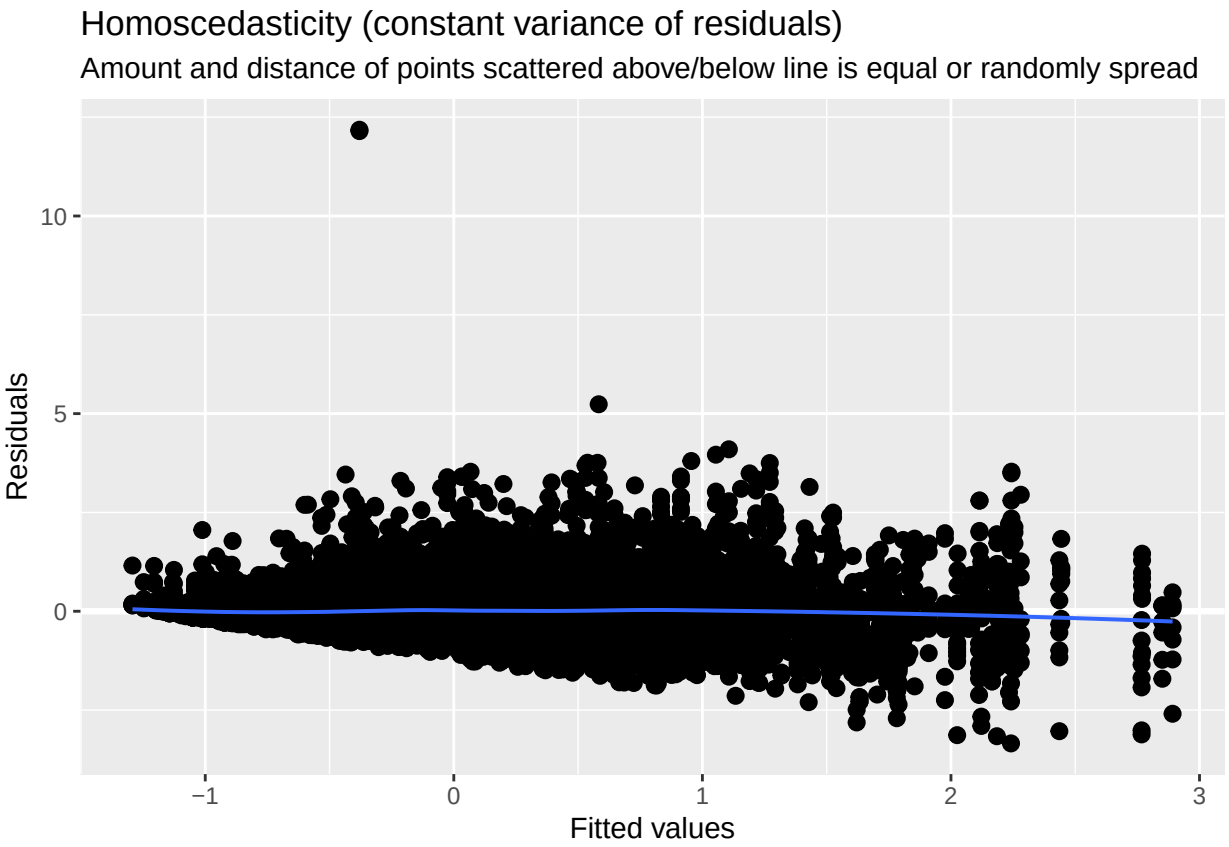

(Intercept)
timepost1
timepost2
timepost3
timepost4
Random effect quantiles
Standard normal quantiles

```
##
##
## [[3]]
```




```
##
## [[4]]

## `geom_smooth()` using formula 'y ~ x'
```



From the plots above, all assumptions are satisfied except the fact that residuals are showing a slight downward trend as fitted values increase. We are looking forward to addressing it in the in-class discussion soon.

Non-parametric Repeated Measures ANOVA

```
x_post1 <- x %>%
  filter(time %in% c("baseline","post1"), lesional == "lesional", paired.pulse == "single")
x_post1 <- x_post1[complete.cases(x_post1), ]
x_post1$time <- factor(x_post1$time, levels=c("baseline","post1"))
#check if the data set is complete
#table(x_post1$time,x_post1$montage,x_post1$subjectID)
```

```
res.aov <- aov(pct.change ~ time*montage + Error(subjectID), data = x_post1)
get_anova_table(res.aov)
```

```
##
## Call:
## aov(formula = pct.change ~ time * montage + Error(subjectID),
##     data = x_post1)
##
## Grand Mean: 4.150957
##
## Stratum 1: subjectID
##
## Terms:
##                 Residuals
## Sum of Squares   167661.7
## Deg. of Freedom        17
```

```
##
## Residual standard error: 99.30989
##
## Stratum 2: Within
##
## Terms:
##                       time   montage time:montage Residuals
## Sum of Squares    14887.1   43969.2      43969.2 1828717.3
## Deg. of Freedom         1         2            2       841
##
## Residual standard error: 46.63106
## Estimated effects may be unbalanced
```

```
# comparisons for montage variable
x_post1 %>%
  pairwise_t_test(
    pct.change ~ montage, paired = TRUE,
    p.adjust.method = "bonferroni"
    )
```

```
## # A tibble: 3 x 10
##   .y.     group1 group2    n1    n2 statistic    df       p   p.adj p.adj.signif
## * <chr>   <chr>  <chr>  <int> <int>     <dbl> <dbl>   <dbl>   <dbl> <chr>
## 1 pct.ch~ anodal bihemi   288   288     -3.76   287 2.09e-4 6.27e-4 ***
## 2 pct.ch~ anodal catho~   288   288     -3.56   287 4.41e-4 1.00e-3 **
## 3 pct.ch~ bihemi catho~   288   288      1.48   287 1.39e-1 4.17e-1 ns
```

```
# comparisons for time variable
x_post1 %>%
  pairwise_t_test(
    pct.change ~ time, paired = TRUE,
    p.adjust.method = "bonferroni"
    )
```

```
## # A tibble: 1 x 10
##   .y.        group1  group2    n1    n2 statistic    df     p p.adj p.adj.signif
## * <chr>      <chr>   <chr>  <int> <int>     <dbl> <dbl> <dbl> <dbl> <chr>
## 1 pct.change baseli~ post1    432   432     -2.52   431 0.012 0.012 *
```

In the pairwise test, both `montage` and `time` are shown to be significant.

Check Repeated Measures ANOVA Assumptions

```
x %>%
  group_by(time) %>%
  identify_outliers(pct.change)
```

Outliers

```
## # A tibble: 657 x 12
##    time  condBySubject amplitude[,1] replicate montage paired.pulse lesional
##    <fct> <chr>                 <dbl> <fct>     <fct>   <fct>        <fct>
##  1 base~ 10232                 2.34  7         bihemi  excitory     nonlesi~
##  2 base~ 11132                 2.61  6         anodal  excitory     nonlesi~
```

```
##  3 base~ 11232              2.20    4         bihemi  excitory     nonlesi~
##  4 base~ 11312              1.84    2         cathod~ single       nonlesi~
##  5 base~ 11332              1.90    1         cathod~ excitory     nonlesi~
##  6 base~ 11332              1.79    4         cathod~ excitory     nonlesi~
##  7 base~ 11332              2.74    6         cathod~ excitory     nonlesi~
##  8 base~ 1212               2.30    8         bihemi  single       nonlesi~
##  9 base~ 12131             -0.00768 7         anodal  excitory     lesional
## 10 base~ 1232               3.80    8         bihemi  excitory     nonlesi~
## # ... with 647 more rows, and 5 more variables: subjectID <fct>,
## #   pct.change <dbl>, BLmeanAmp <dbl>, is.outlier <lgl>, is.extreme <lgl>
```

There are quite a few extreme outliers in `pct.change` (e.g. a percent change of 617.4672). Thus, the assumption that there were no extreme outliers is violated.

```
x %>%
  group_by(time) %>%
  shapiro_test(pct.change)
```

Normality

```
## # A tibble: 5 x 4
##   time     variable   statistic        p
##   <fct>    <chr>          <dbl>    <dbl>
## 1 baseline pct.change     0.741 3.38e-53
## 2 post1    pct.change     0.774 6.98e-51
## 3 post2    pct.change     0.466 2.39e-66
## 4 post3    pct.change     0.809 8.25e-48
## 5 post4    pct.change     0.654 2.64e-58
```

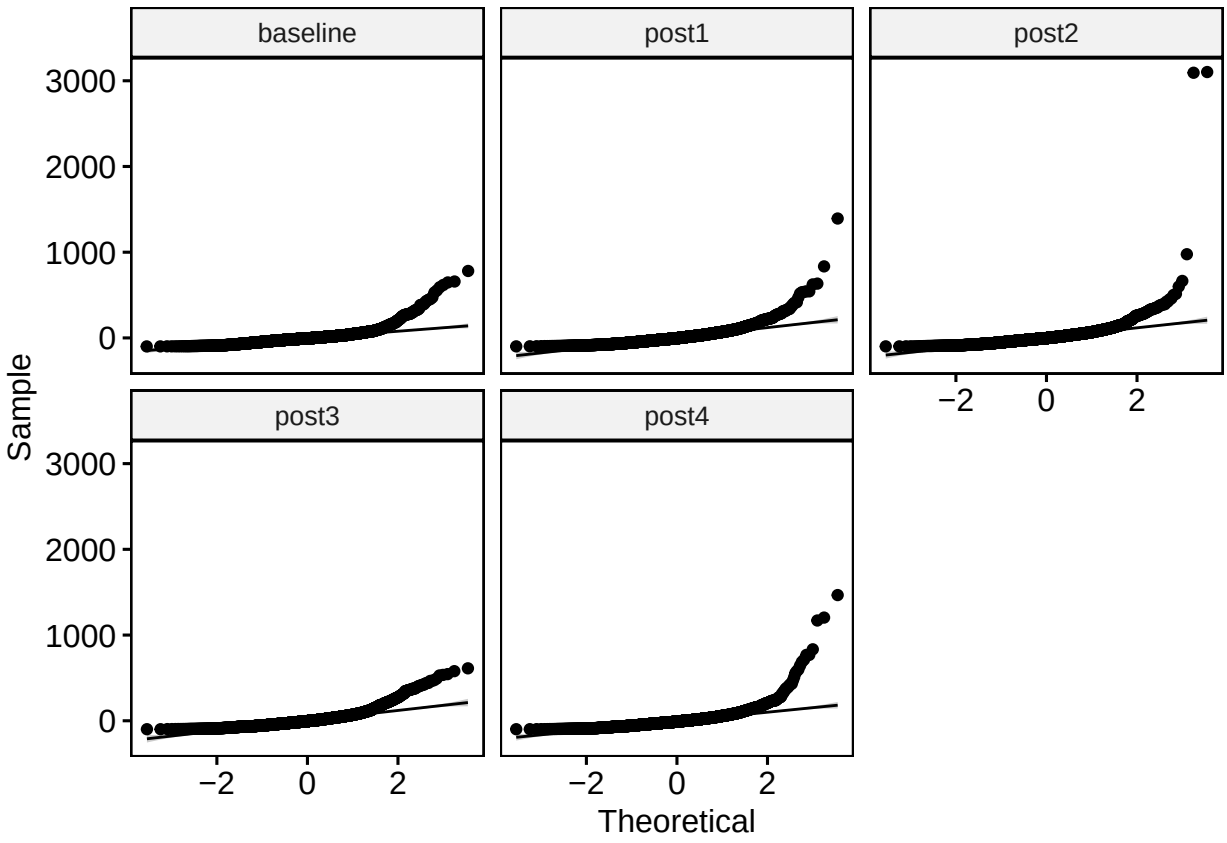
According to the Shapiro-Wilk's test, since the observed p-values for all categories in `time` are less than 0.05, we reject the null hypothesis that the data are normally distributed.

```
ggqqplot(x, "pct.change", facet.by = "time")
```

```
## Warning: Removed 50 rows containing non-finite values (stat_qq).
```

```
## Warning: Removed 50 rows containing non-finite values (stat_qq_line).
```

```
## Warning: Removed 50 rows containing non-finite values (stat_qq_line).
```



According to the QQ-plot, in all categories in `time`, the larger points deviate significantly from the reference line. Thus, we conclude that the normality assumption is violated.