UC Irvine ISI-BUDS 2023 Day 10: Mixed-Effects Models

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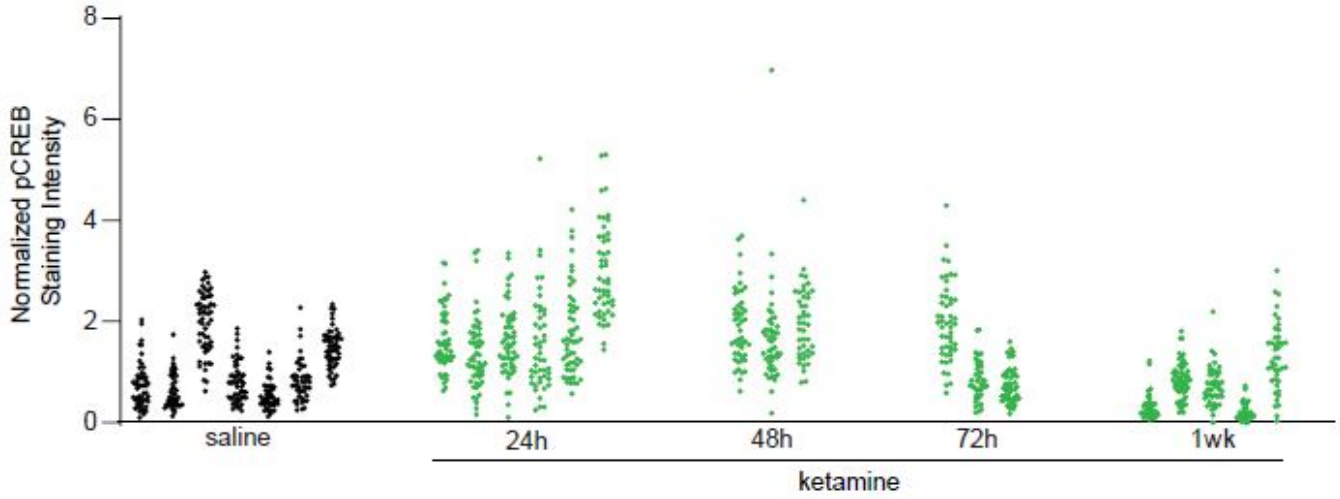
Learning Objectives

- Motivating Example
- LM, LME, GLM, and GLMM
- LME Examples: Examples 1 - 3
- Generalized Linear Mixed-Effects Model (GLMM): Example 4
- The slides are based on my published work: https://doi.org/10.1016/j.neuron.2021.10.030 https://yu-zhaoxia.github.io/MM_in_Neuroscience/

Motivating Example

Example 1: Data

- 1200 neurons from 24 mice; 5 conditions/groups



Example 1: Data

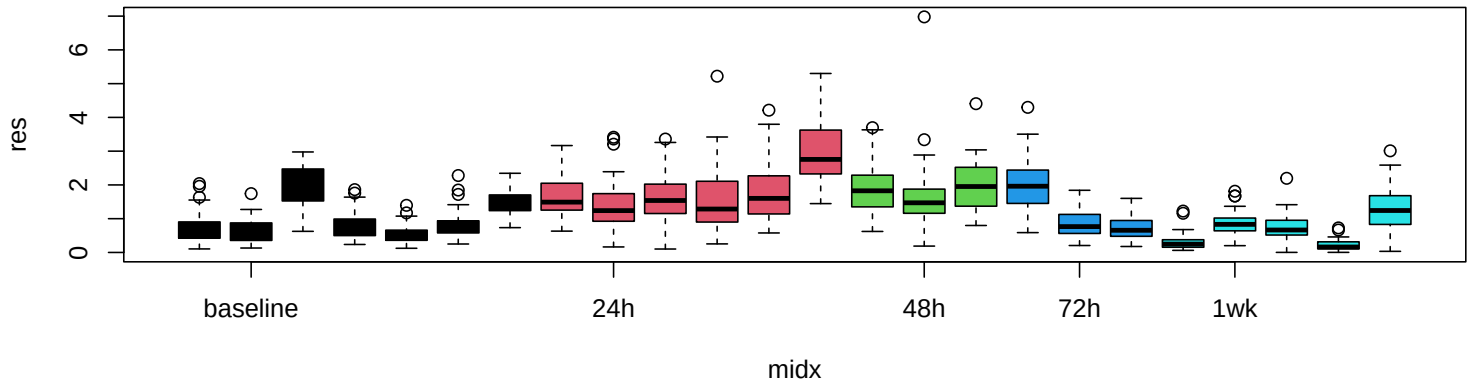
```
Ex1=read.csv("https://www.ics.uci.edu/~zhaoxia/Data/BeyondTandANOVA/Example1.txt", head=T)

#Do not forget to factor the treatment IDs and animal IDs
#This is particularly important for the treatment_idx,
#else the values will be treated as numerical values, rather than levels
Ex1$treatment_idx = as.factor(Ex1$treatment_idx)
Ex1$midx = as.factor(Ex1$midx)
head(Ex1)
```

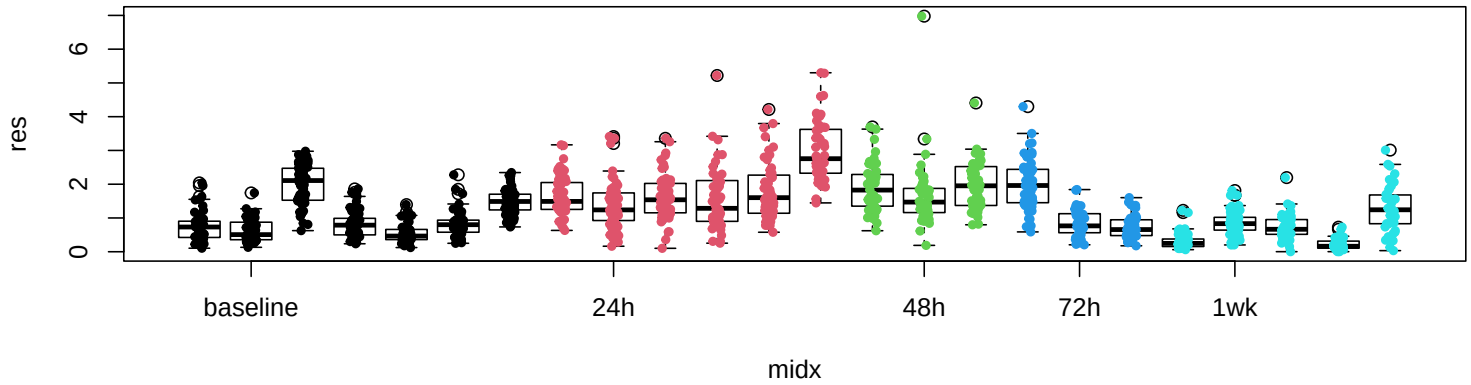
```
##         res treatment_idx midx
## 1 1.6326840             1    1
## 2 0.9698389             1    1
## 3 0.5184931             1    1
## 4 0.3031273             1    1
## 5 0.5815271             1    1
## 6 0.5001287             1    1
```

Example 1: Data Visualization

- boxplots by R base graphics

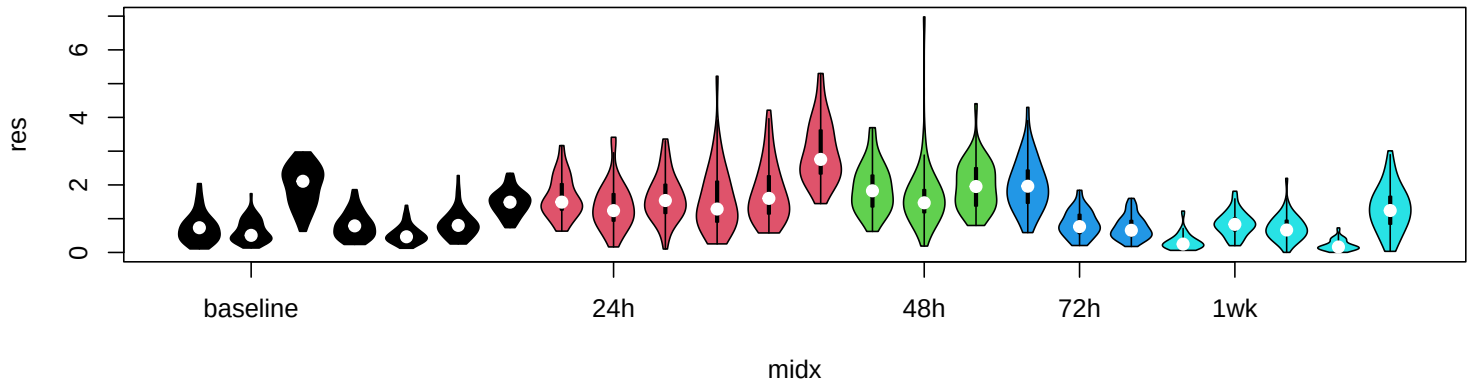
```
#Use base graphics
mycolors=rep(1:5, c(7,6,3,3,5)) #different colors for different treatment groups

#a basic plot of boxplots by mice
#Mice in the same treatment groups use the same color
boxplot(res~midx, data=Ex1, col=mycolors, xaxt="n")
axis(1, at = 1+c(1, 8, 14, 17, 20),
     labels = c("baseline", "24h", "48h", "72h", "1wk"))

#boxplot with jitter
boxplot(res~midx, data=Ex1, col=0, xaxt="n")
axis(1, at = 1+c(1, 8, 14, 17, 20),
     labels = c("baseline", "24h", "48h", "72h", "1wk"))
stripchart(res ~ midx, vertical = TRUE, data = Ex1,
           method = "jitter", add = TRUE, pch = 20, col = mycolors)
```

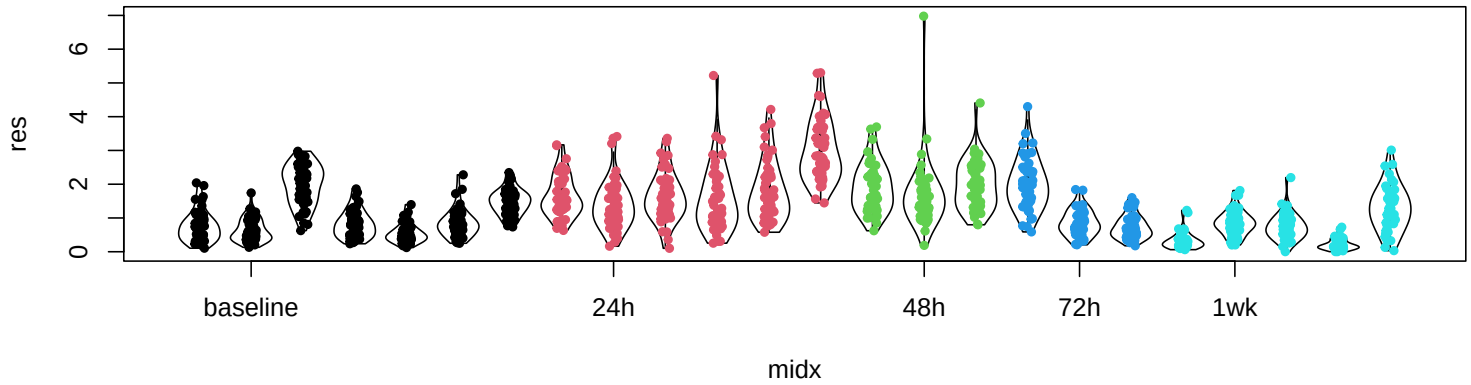

res
0
2
4
6
baseline
24h
48h
72h
1wk
midx


res
0
2
4
6
baseline
24h
48h
72h
1wk
midx

Violin plots generated by the vioplot package

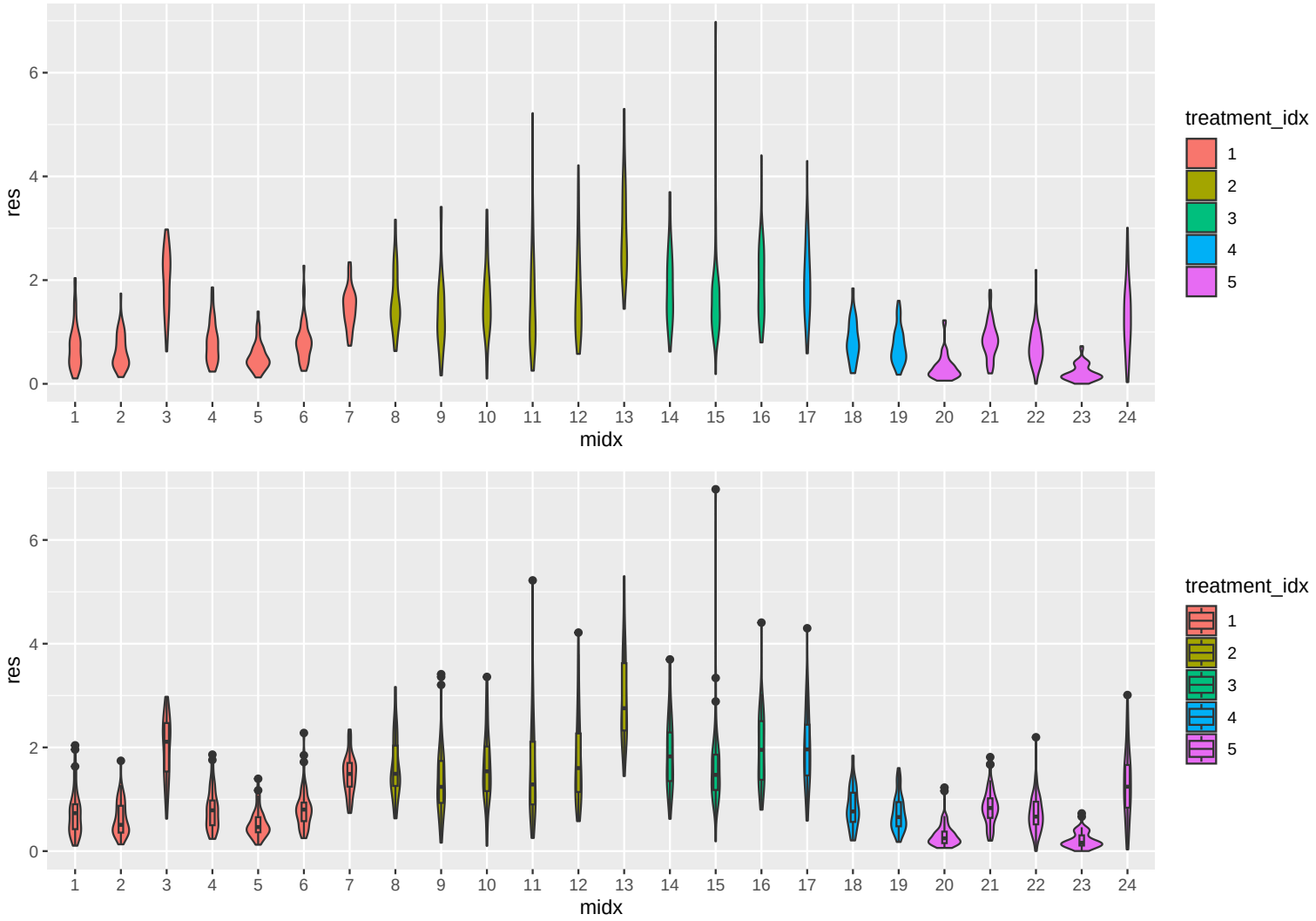
```
par(mfrow=c(2,1)) #split the plot window to two vertically-stacked ones
vioplot(res~midx, data=Ex1, col=mycolors, xaxt = "n")
axis(1, at = 1+c(1, 8, 14, 17, 20),
     labels = c("baseline", "24h", "48h", "72h", "1wk"))

#violin plot with jitters
vioplot(res~midx, data=Ex1, col=0, xaxt="n")
stripchart(res ~ midx, vertical = TRUE, data = Ex1,
           method = "jitter", add = TRUE, pch = 20, col = mycolors)
axis(1, at = 1+c(1, 8, 14, 17, 20),
     labels = c("baseline", "24h", "48h", "72h", "1wk"))
```


res
0
2
4
6
baseline
24h
48h
72h
1wk
midx


res
0
2
4
6
baseline
24h
48h
72h
1wk
midx

Fancy plots generated by ggplot2 package

```
plot1=ggplot(Ex1, aes(x = midx, y = res, fill=treatment_idx)) +
  geom_violin()
#boxplot within violin plot
plot2=ggplot(Ex1, aes(x = midx, y = res, fill=treatment_idx)) +
  geom_violin()+
  geom_boxplot(width=0.1)
grid.arrange(plot1, plot2, ncol=1, nrow=2)#library(gridExtra)
```



Example 1: The "Familiar" Analysis

```
summary(aov(res~treatment_idx, data=Ex1))
```

```
##                 Df Sum Sq Mean Sq F value Pr(>F)
## treatment_idx    4  246.6   61.66   108.1 <2e-16 ***
## Residuals     1195  681.6    0.57
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
summary(lm(res~treatment_idx, data=Ex1))
```

```
##
## Call:
## lm(formula = res ~ treatment_idx, data = Ex1)
##
## Residuals:
##     Min      1Q  Median      3Q     Max
## -1.7076 -0.5283 -0.1801  0.3816  5.1378
##
## Coefficients:
##                Estimate Std. Error t value Pr(>|t|)
## (Intercept)     1.02619    0.03997  25.672  < 2e-16 ***
## treatment_idx2  0.78286    0.05868  13.340  < 2e-16 ***
## treatment_idx3  0.81353    0.07551  10.774  < 2e-16 ***
## treatment_idx4  0.16058    0.07349   2.185   0.0291 *
## treatment_idx5 -0.36047    0.06266  -5.753 1.11e-08 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.7553 on 1195 degrees of freedom
```

Example 1: The "Familiar" Approach for Null Data

- Is the familiar approach valid? We evaluate the method using data generated under the null hypothesis
- We can generate a null data set by permuting the treatment group labels of the animals
- We generate 1000 null data sets and check how many times the familiar approach will reject the null hypothesis of no group difference

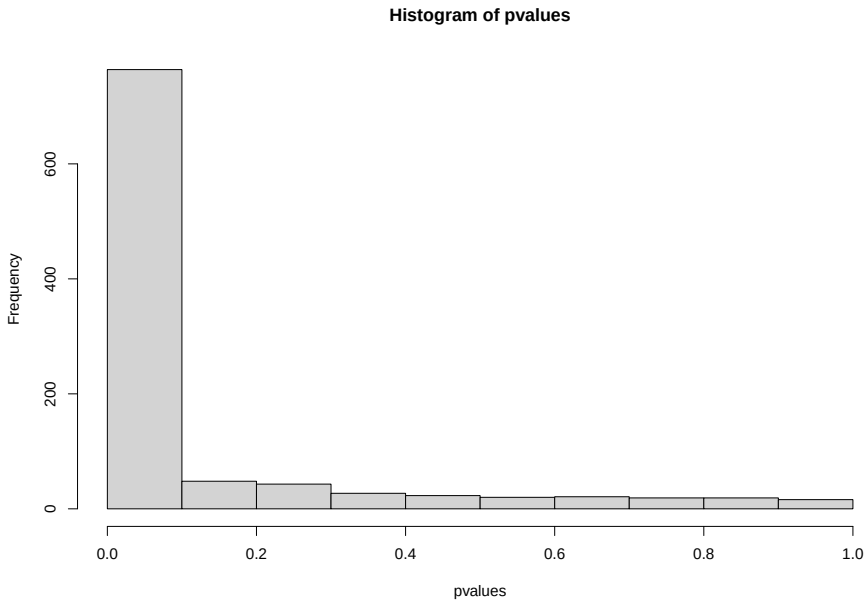
Example 1: The "Familiar" Approach for Null Data

```
treatment=rep(1:5, c(7,6,3,3,5))
ncell=sapply(split(Ex1, Ex1$midx), dim)[1,]
#generate pseudo (permuted) 1000 times by randomly
#shuffling the treatment labels across mice
pvalues=rep(NA, 1000)#initialize a vector of p-values
for(i in 1:1000) {
  Ex1.perm = data.frame(res=Ex1$res,
                        treatment_idx=rep(sample(treatment),ncell), midx=Ex1$midx)
  pvalues[i]=summary(lm(res~treatment_idx, data=Ex1.perm))$coefficients[2,4] }
```

Example 1: P-values using 1000 Null Data sets

- What does the histogram suggest?

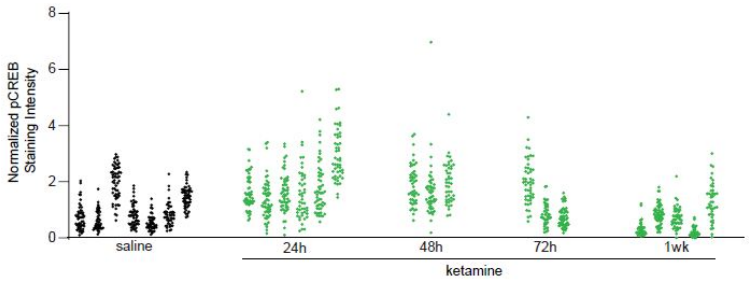
```
hist(pvalues)
```



Why does LM fail for Example 1?

- This because the observations are not independent
- We can compute Intra-Class Correlation (ICC) to quantify the magnitude of clustering due to animal effects.

	Saline (7 mice)	24h (6 mice)	48h (3 mice)	72h (3 mice)	1wk (5 mice)
# of cells	357.0000000	309.0000000	139.000000	150.000000	245.0000000
ICC	0.6209487	0.3300633	0.017803	0.628109	0.5369458



ICC Analysis of Example 1

- The ICC indicates that the dependency due to clustering is substantial.
- Therefore, the 1,200 neurons should not be treated as 1,200 independent cells.
- When dependence is not adequately accounted for, the type I error rate can be much higher than the pre-chosen level of significance.

From LM to LME

LM (incorrect!) for Example 1

- Consider Example 1. Let
  - Y_{ij} indicate the jth observed response of the ith mouse.
  - x_{ij} be the treatment label, with $x_{ij} = 1$ for baseline, $x_{ij} = 2$ for 24 hours, $x_{ij} = 3$ for 48 hours, $x_{ij} = 4$ for 72 hours, and $x_{ij} = 5$ for 1 week after ketamine treatments.
- In the inner mathematical computation, four dummy variables, which take value 0 or 1, are generated: $x_{ij,1} = 1$ for 24 hours, $x_{ij,2} = 1$ for 48 hours, $x_{ij,3} = 1$ for 72 hours, and $x_{ij,4} = 1$ for 1 week after ketamine treatments, respectively.

$$Y_{ij} = \beta_0 + x_{ij,1}\beta_1 + \ldots + x_{ij,4}\beta_4 + \epsilon_{ij},$$
$$i = 1, \ldots, 24; j = 1, \ldots, n_i;$$

where n_i is the number of observations from the ith mouse.

LME for Example 1

- The 1200 observations are clustered by animal. We account for the resulting dependence by adding an animal specific effect, as follows:

$$Y_{ij} = \beta_0 + x_{ij,1}\beta_1 + \ldots + x_{ij,4}\beta_4 + u_i + \epsilon_{ij},$$
$$i = 1, \ldots, 24; j = 1, \ldots, n_i;$$

where

- u_i indicates the deviance between the overall intercept β_0 and the mean specific to the ith mouse
- ϵ_{ij} represents the deviation in pCREB immunoreactivity of observation (cell) j in mouse i from the mean pCREB immunoreactivity of mouse i
- $(\beta_0, \beta_1, \beta_2, \beta_3, \beta_4)$ are assumed to be fixed but unknown
- $(u_1, \cdots, u_{24})$ are treated as independent and identically distributed random variables from a normal distribution with mean 0 and a variance parameter that reflects the variation across animals.

LME for Example 1

- Similar to the treatment variable, for the animal ID variable, the users do not need to define the dummy variables, which are generated by R automatically in its inner working.
- Thus, equivalently, one could write the previous equation by using a vector $(z_{ij,1}, \ldots, z_{ij,24})$ of dummy variables for the cluster/animal memberships such that $z_{ij,k} = 1$ for $i = k$ and 0 otherwise:

$$Y_{ij} = \beta_0 + x_{ij,1}\beta_1 + \ldots + x_{ij,4}\beta_4 + z_{ij,1}u_1 + \ldots + z_{ij,24}u_{24} + \epsilon_{ij},$$
$$i = 1, \ldots, 24; j = 1, \ldots, n_i;$$

LME for Example 1

- Y_{ij} is modeled by three components:
  - the fixed-effects from the covariates $(x_{xij,1}, \ldots, x_{ij,4})$ and the overall intercept β_0, which is the population mean of the reference group in this example
  - the random-effects due to the clustering $(z_{ij,1}, \ldots, z_{ij,24})$
  - the random errors ϵ_{ij}'s

R Packages for LME

- Two major packages are 'nlme' and 'lme4'.
- Syntax:
  - 'nlme::lme(res~treatment_idx, data= Ex1, random = ~ 1|midx)'
  - 'lme4::lmer(res ~ treatment_idx+(1|midx), data=Ex1)'
- Note that, similar to the fixed effects, for the random-effects, we don't need to created the dummy variables. This will be done internally by R.
- For the fixed-effects (treatment_idxhere), make sure that it is a factor, not numerical, as the levels "1-5" denote different times points
- For the random-effects from "midx"(mice), R treated it as a factor with different levels (animals)

LM, LME, GLM, and GLMM

LM and LME: Matrix Format

- LM: $Y = X\beta + \epsilon$
  - a linear predictor $X\beta$
  - random errors ϵ are independent, have a zero mean and a constant variance.
  - $\epsilon \sim N(0, \sigma^2\mathbf{I})$ is used for deriving t- and F-tests. Typically this assumption is not very critical as long as the sample size is not too small
- LME: $Y = X\beta + Z\mathbf{u} + \epsilon$
  - fixed-effects: a linear predictor $X\beta$
  - random-effects: $Z\mathbf{u}$, where $\mathbf{u} \sim N(0, G)$. E.g., $G = \sigma_b^2\mathbf{I}$.
  - random errors: $\epsilon \sim N(0, \sigma^2\mathbf{I})$, independent with $\mathbf{u}$.

GLM

- The components of GLM:
  - a linear predictor $X\beta$
  - a link function to connect $E(Y|X)$ and $X\beta$: $g(E(Y|X)) = X\beta$
  - a distribution for Y given $E(Y|X)$
- The components of GLMM:
  - fixed-effects: a linear predictor $X\beta$
  - random-effects: $Z\mathbf{u}$, where $\mathbf{u} \sim N(0, G)$. E.g., $G = \sigma_b^2\mathbf{I}$.
  - a link function to connect $E(Y|X, \mathbf{u})$ and $X\beta$: $g(E(Y|X, \mathbf{u})) = X\beta + Z\mathbf{u}$
  - a distribution for Y given $E(Y|X)$

LME Examples: Example 1

LME Examples: Example 2

```
# The nlme:lme function specifies the fixed effects in the formula
# (first argument) of the function, and the random effects
# as an optional argument (random=). The vertical bar | denotes that
# the cluster is done through the animal id (midx)
obj.lme=lme(res~treatment_idx, data= Ex1, random = ~ 1|midx, method="ML")
summary(obj.lme)$tTable
```

```
##                     Value Std.Error   DF    t-value      p-value
## (Intercept)     1.0008500 0.1750995 1176  5.7158919 1.382236e-08
## treatment_idx2  0.8191952 0.2577129   19  3.1787124 4.944475e-03
## treatment_idx3  0.8427397 0.3200466   19  2.6331777 1.638113e-02
## treatment_idx4  0.1896571 0.3197681   19  0.5931081 5.601033e-01
## treatment_idx5 -0.3202969 0.2713859   19 -1.1802269 2.524757e-01
```

- The results from LME is more realistic

```
summary(obj.lme)
```

```
## Linear mixed-effects model fit by maximum likelihood
##   Data: Ex1
##        AIC      BIC    logLik
##   2272.961 2308.592 -1129.481
##
## Random effects:
##  Formula: ~1 | midx
##         (Intercept)  Residual
## StdDev:   0.4545821 0.5995347
##
## Fixed effects:  res ~ treatment_idx
##                     Value Std.Error   DF   t-value p-value
## (Intercept)     1.0008500 0.1750995 1176  5.715892  0.0000
## treatment_idx2  0.8191952 0.2577129   19  3.178712  0.0049
## treatment_idx3  0.8427397 0.3200466   19  2.633178  0.0164
## treatment_idx4  0.1896571 0.3197681   19  0.593108  0.5601
## treatment_idx5 -0.3202969 0.2713859   19 -1.180227  0.2525
##  Correlation:
##                (Intr) trtm_2 trtm_3 trtm_4
## treatment_idx2 -0.679
## treatment_idx3 -0.547  0.372
## treatment_idx4 -0.548  0.372  0.300
## treatment_idx5 -0.645  0.438  0.353  0.353
##
## Standardized Within-Group Residuals:
##        Min         Q1        Med         Q3        Max
## -2.5410173 -0.5737059 -0.1133680  0.4733263  8.8578521
##
## Number of Observations: 1200
## Number of Groups: 24
```

```
anova(obj.lme)
```

```
##                numDF denDF   F-value p-value
## (Intercept)        1  1176 179.66421  <.0001
## treatment_idx      4    19   5.89455  0.0029
```

LME Examples: Example 2

LME Examples: Example 2

- Research question: determine how in vivo calcium (Ca++) activity of PV cells (measured longitudinally by the genetically encoded Ca++ indicator GCaMP6s) changes over time after ketamine treatment
- Study: Ca++ event frequencies were measured at 24h, 48h, 72h, and 1 week after ketamine treatment in four mice
- Want to compare Ca++ event frequency at 24h to the other three time points.
- In total, Ca++ event frequencies of 1,724 neurons were measured.

Example 2: Data

```
library(nlme)
library(lme4)
library(lmerTest)
Ex2=read.csv("https://www.ics.uci.edu/~zhaoxia/Data/BeyondTandANOVA/Example2.txt", head=T)
Ex2$treatment_idx=Ex2$treatment_idx-4
Ex2$treatment_idx=as.factor(Ex2$treatment_idx)
### covert the variable of mouse IDs to a factor
Ex2$midx=as.factor(Ex2$midx)
```

Example 2: Wrong analysis

```
lm.obj=lm(res~treatment_idx, data=Ex2)
summary(lm.obj)$coefficients
```

```
##                   Estimate Std. Error    t value     Pr(>|t|)
## (Intercept)     0.71490545 0.01233741 57.9461618 0.000000e+00
## treatment_idx2 -0.07802047 0.01701121 -4.5864155 4.835037e-06
## treatment_idx3  0.00914741 0.01718859  0.5321791 5.946707e-01
## treatment_idx4  0.04971562 0.01633230  3.0440051 2.369903e-03
```

Example 2: Wrong analysis

- The LM (including ANOVA, t-test) analysis results indicate
  - significantly reduced Ca++ activity at 48h relative to 24h with $p = 4.8 \times 10^{-6}$
  - significantly increased Ca++ activity at 1week compared to 24h with $p = 2.4 \times 10^{-3}$
  - However, if we account for repeated measures due to cells clustered in mice using LME, the changes are no longer significant

Example 2: LME

```
lmer.obj=lmerTest::lmer(res~treatment_idx+(1|midx), data= Ex2, REML="FALSE")
summary(lmer.obj)$coefficients
```

```
##                    Estimate Std. Error          df    t value     Pr(>|t|)
## (Intercept)     0.699786009 0.03484986    4.901964 20.0800262 6.756672e-06
## treatment_idx2 -0.017490109 0.01726513 1723.485832 -1.0130306 3.111877e-01
## treatment_idx3  0.009353984 0.01657856 1720.292658  0.5642219 5.726767e-01
## treatment_idx4  0.029448530 0.01656107 1719.621372  1.7781780 7.555129e-02
```

Example 2: LM vs LME

Estimated changes of Ca+ event frequency (the baseline is 24h after treatment)

	48h	72h	1wk
LM est	-0.078 $\pm$.017	0.009$\pm$0.017	0.050$\pm$0.016
LM p	4.8×10^{-6}	0.595	2.4×10^{-3}
LME est	-0.017$\pm$0.017	0.009$\pm$0.017	0.029$\pm$0.017
LME p	0.311	0.573	0.076

Pooling data naively is not a good idea

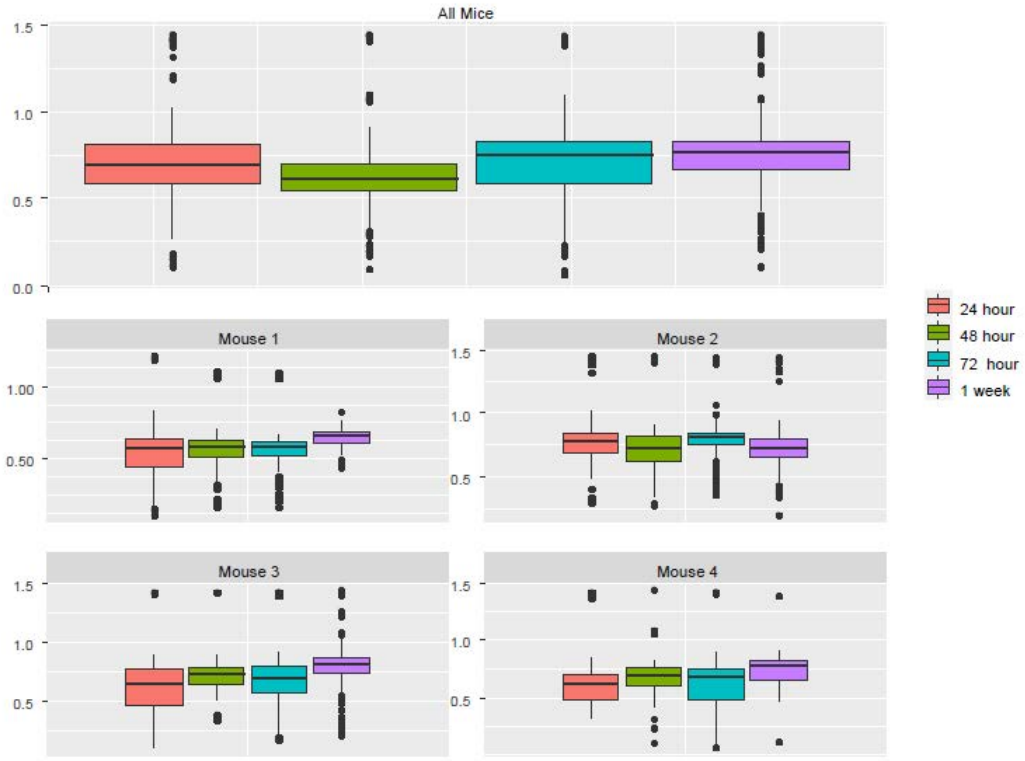


Figure 1: The boxplots of Ca++ event frequencies measured at four time points. (A) Boxplot of Ca++ event frequencies using the pooled neurons from four mice. (B) boxplots of Ca++ event frequencies stratified by individual mice.

Pooling data naively is not a good idea

- Consider the change in Ca++ activities from 24h to 48h
- Pooled data from all mice:
  - The box plots suggest reduction in Ca++ activities
- Individual mice data:
  - The box plots of Mouse 2 suggest a noticeable reduction
  - However, there was almost no change in Mouse 1
  - Mouse 3 and Mouse 4 might suggest small increases, rather than decreases

Pooling data naively is not a good idea

- Why do the pooled data follow the pattern of Mouse 2?

	24h	48h	72h	1wk	Total
Mouse 1	81	254	88	43	466(27%)
Mouse 2	206	101	210	222	739 (43%)
Mouse 3	33	18	51	207	309 (18%)
Mouse 4	63	52	58	37	210 (12%)
Total	383	425	407	509	1,724 (100%)

- Mouse 2 contributed 43% cells!

Remark: on the minimum number of levels for using random-effects

- In Example 2, the number of levels in the random-effects variable is four, as there are four mice.
- According to Gelman and Hill 2006, it does not hurt to use random-effects in this situation.
- There is no unique answer on the minimum number of levels for using random-effects.

Remark: on the minimum number of levels for using random-effects

- An alternative is to include the animal ID variable as factor with fixed animal effects.
- Neither of two approaches is the same as fitting an LM to the pooled cells naively.
- In a more extreme case, for an experiment using only two monkeys for example,
  - naively pooling data (such as neurons) is NOT recommended.
  - a more appropriate approach is to analyze the animals separately and then check whether the results from the two animals are consistent

LME Examples: Example 3

Example 3: Data Structure

- Ca++ event integrated amplitudes are compared between baseline and 24h after ketamine treatment.
- 1244 cells were sampled from 11 mice
- each cell was measured twice (baseline and after ketamine treatment)
- correlation arises from both cells and animals, which creates a three-level structure:
  - measurements within cells and cells within animals.

```
library(nlme)
library(lme4)
library(lmerTest)
Ex3=read.csv("https://www.ics.uci.edu/~zhaoxia/Data/BeyondTandANOVA/Example3.txt", head=T)
```

Example 3: LM vs LME

```
#### wrong analysis: using the linear model
summary(lm(res~treatment, data=Ex3[!is.na(Ex3$res),])) #0.0036
#### wrong analysis using t tests (paired or unpaired)
t.test(Ex3[Ex3$treatment==1,"res"], Ex3[Ex3$treatment==2,"res"], var.eq=T)
t.test(Ex3[Ex3$treatment==1,"res"], Ex3[Ex3$treatment==2,"res"])
t.test(Ex3[Ex3$treatment==1,"res"], Ex3[Ex3$treatment==2,"res"], paired=T)

#LME
lme.obj1=lme(res~ treatment, random =~1| midx/cidx,
             data= Ex3[!is.na(Ex3$res),], method="ML")
summary(lme.obj1)
```

Example 3: LM vs LME

- LME and LM produce similar estimates for the fix-effects coefficients
- the standard error of the LM is larger; the p-value based on LME is smaller (0.0036 for LM vs 0.0001 for LME).
- In this example, since the two measures from each cell are positively correlated, the variance of the differences is smaller when treating the data as paired rather than independent.
- As a result, LME produces a smaller p-value
- Rigorous statistical analysis is not a hunt for the smallest p value (commonly known as p-hacking or significance chasing)

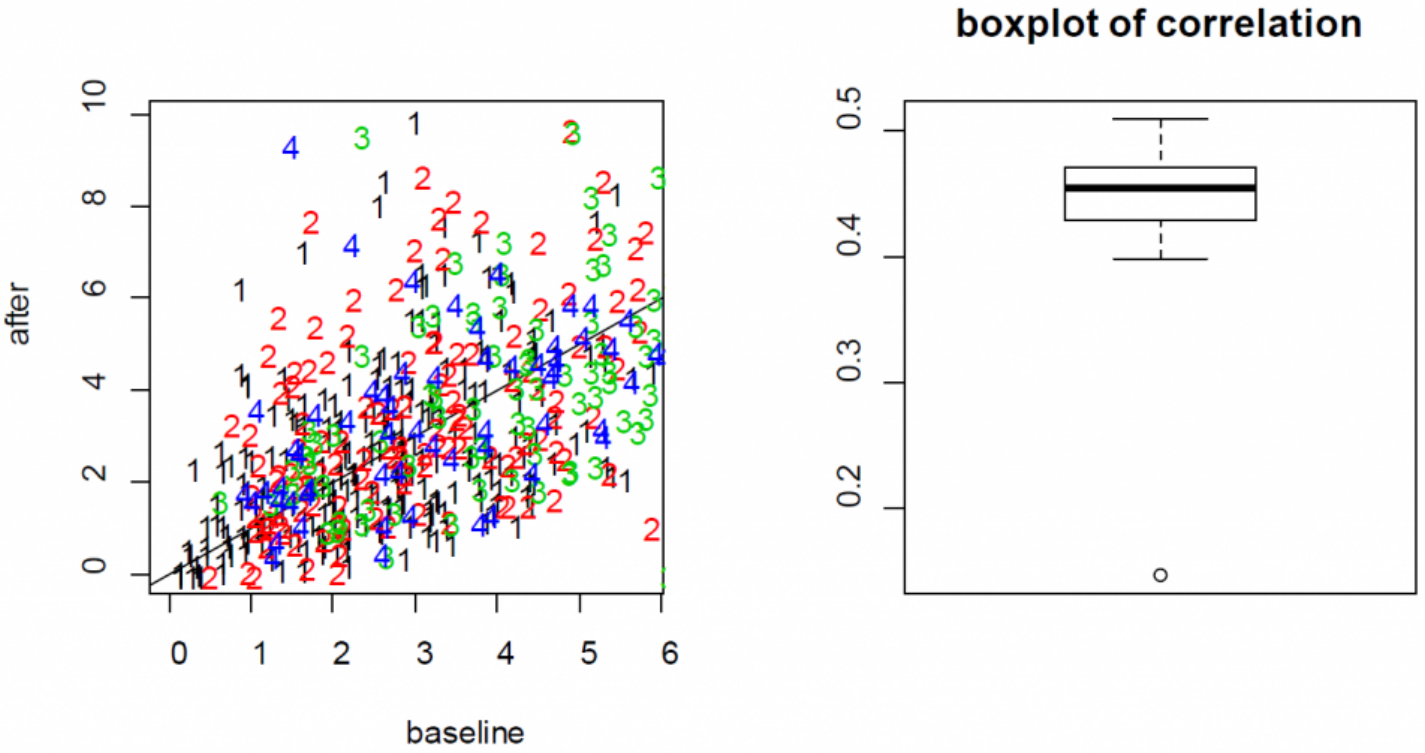


Figure 2: (Left) the scatter plot of Ca++ event integrated amplitude at baseline vs 24h after treatment for the neurons from four mice (labeled as 1, 2, 3 and 4) indicates that the baseline and after-treatment measures are positively correlated. (Right) boxplot of the baseline and after-treatment correlations of the 11 mice.

A note on "nested" random effects

- When specifying the nested random effects, we used "random =~1| midx/cidx".
- This leads to random effects at two levels: the mouse level and the cells-within-mouse level.
- This specification is important if same cell IDs might appear in different mice.
- When each cell has its unique ID, just like "cidx" variable in Example 3, it does not matter and "random =list(midx=~1, cidx=~1)" leads to exactly the same model.

A note on "nested" random effects

```
### to verify that the cell IDs are indeed unique
length(unique(Ex3$cidx))
#lme.obj2 is the same as lme.obj
lme.obj2=lme(res~ treatment, random =list(midx=~1, cidx=~1), data=Ex3[!is.na(Ex3$res),], method="ML")
summary(lme.obj2)
```

On models with more random effects

- The above LME model only involves random intercepts.
- There might be random effects due to multiple sources.
- A model with more random-effects might be a better choice.
- Visualization is a useful exploratory tool to help identify an appropriate model.

On models with more random effects

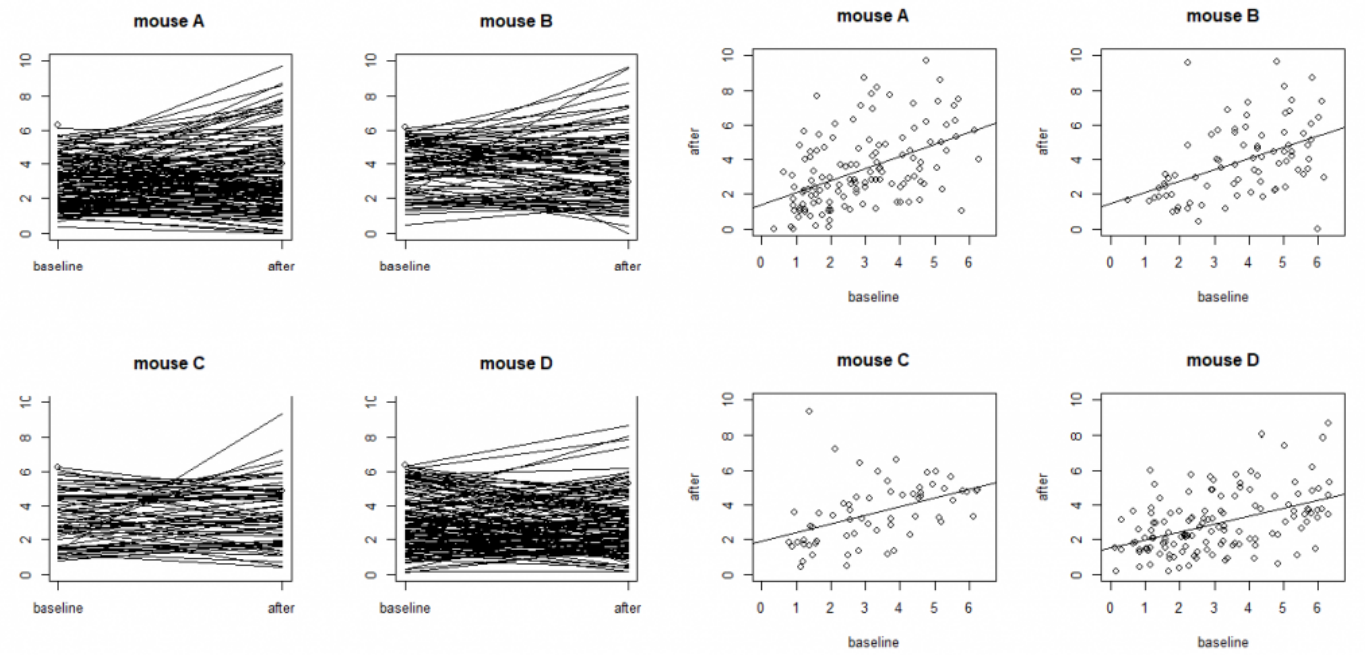


Figure 3: Ca++ event integrated amplitudes at baseline vs 24h after treatment for the neurons from four mice (labeled as A, B, C and D) with each dot representing a neuron. The four plots on the left are "spaghetti" plots of the four animals with each line representing the values at baseline and 24h after treatment for a neuron; the four plots on the right report the before-after scatter plots (with fitted straight lines using a simple linear regression)

Compare Models with Different Random Effects

- Skipped. See Example 3 of https://yu-zhaoxia.github.io/MM_in_Neuroscience/

On models with more random effects

- Tests can be used to compare models with different random effects
  - Need to be careful. See 6.4 of https://yu-zhaoxia.github.io/MM_in_Neuroscience/
- For example 3, the model I chose have the following random-effects:

"random=list(midx=~1, cidx=~treatment)"

- It improves lme.obj1 substantially.
- Adding more random-effects does not lead to further improvement

GLMM: Example 4

Generalized Linear Mixed-Effects Model (GLMM)

- The components of aGLMM:
  - fixed-effects: a linear predictor $X\beta$
  - random-effects: $Z\mathbf{u}$, where $\mathbf{u} \sim N(0, G)$. E.g., $G = \sigma_b^2 \mathbf{I}$.
  - a link function to connect $E(Y|X, \mathbf{u})$ and $X\beta$:

$$g(E(Y|X, \mathbf{u})) = X\beta + Z\mathbf{u}$$

  - a distribution for Y

GLMM Examples: A Simulated Data Set

- The simulation used parameters estimated from real data
- Eight mice were trained to do task
- The behavior outcome is whether the animals make the correct predictions
  - 512 trials in total: 216 correct trials, 296 wrong trials
- Mean neuronal activity levels (dF/F) were recorded for each trial
- We would like to model behaviors using neuronal data (decoding)

Use lme4::glmer to fit a GLMM

```
library(lme4)
library(pbkrtest)
waterlick=read.table("https://www.ics.uci.edu/~zhaoxia/Data/BeyondTandANOVA/waterlick_sim.txt", head=T)
summary(waterlick)
```

```
##     mouseID          lick             dff          
##  Min.   :1.000   Min.   :0.0000   Min.   :-8.838  
##  1st Qu.:2.000   1st Qu.:0.0000   1st Qu.: 1.240  
##  Median :4.500   Median :0.0000   Median : 4.702  
##  Mean   :4.527   Mean   :0.4219   Mean   : 4.810  
##  3rd Qu.:6.000   3rd Qu.:1.0000   3rd Qu.: 8.426  
##  Max.   :8.000   Max.   :1.0000   Max.   :20.456
```

```
#change the mouseID to a factor
waterlick[,1]=as.factor(waterlick[,1])
```

Use lme4::glmer to fit a GLMM

```
obj.glmm=glmer(lick~dff+(1|mouseID),
data=waterlick,family="binomial")
#summary(obj.glmm)
#compute increase in odds and a 95% CI
exp(c(0.06235, 0.06235-1.96*0.01986, 0.06235+1.96*0.01986))-1
```

```
## [1] 0.06433480 0.02370091 0.10658157
```

Interpret GLMM results

- The estimate of odd is 6.4% increase and a 95% confidence interval is 2.3% to 10.7%
- The interpretation of the fixed effects for GLMM is complicated by both
  - the random effects and
  - non-linear link functions
- Among typical mice, the odds of making correct licks increased by 6.4% (95% C.I.: 2.4%-10.7%) with one unit increase in dF/F.

LRT test

- Likelihood ratio test can be done by comparing the model with and the model without the "dff" variance (neuronal activity). Large-sample approximation is used.

```
#fit a smaller model, the model with the dff variable removed
obj.glmm.smaller=glmer(lick~(1|mouseID),
data=waterlick,family="binomial")
#use the anova function to compare the likelihoods of the two models
anova(obj.glmm, obj.glmm.smaller)
#alternatively, one can use the "drop1" function to test the effect of dfff
drop1(obj.glmm, test="Chisq")
```

Improve accuracy of p-values

- The large-sample approximations in GLMM might not be accurate
- We show how to conduct a parametric bootstrap test

```
#The code might take a few minutes
PBmodcomp(obj.glmm, obj.glmm.smaller)
```

- By default, 1000 samples were generated to obtain an empirical null distribution of the likelihood ratio statistic

Convergence Issues

- GLMM is harder to converge than LME.
  - Increase the number of iterations
  - Switch to a different numerical maximization methods
  - Modify models such as eliminate some random effects

https://rstudio-pubs-static.s3.amazonaws.com/33653_57fc7b8e5d484c909b615d8633c01d51.html

https://bbolker.github.io/mixedmodels-misc/glmmFAQ.html

https://m-clark.github.io/posts/2020-03-16-convergence/

Convergence Issues

- Consider more robust methods such generalized estimating equation (GEE)
- Oftentimes, Bayesian approaches are easier to converge