# UC Irvine ISI-BUDS Day 14

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# Study Goals

- LM, LME, GLM, and GLMM
- LME Examples: Example 2
- LME Examples: Example 3
- Generalized Linear Mixed-Effects Model (GLMM)
- 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/

# LM, LME, GLM, and GLMM

# LM and its Matrix Form

- The model

$$Y_i = \beta_0 + x_{i1} \times \beta_1 + \ldots + x_{ip} \times \beta_p + \epsilon_i, i = 1, \ldots, n$$

- Assumptions
  - $E(\epsilon_i) = 0$, which is equivalent to $E(Y_i|X_i) = \beta_0 + x_{i1} \times \beta_1 + \ldots + x_{ip} \times \beta_p$
  - $Var(\epsilon_i) = \sigma^2$, which is equivalent to $Var(Y_i|X_i) = \sigma^2$.
  - $(\epsilon_1, \cdots, \epsilon_n)$ are mutually independent
- The equivalent matrix form is $Y = X\beta + \epsilon$, where the random errors have a zero mean and a common variance, and are independent with each other

# LM and LME

- The components of LM: $Y = X\beta + \epsilon$
  - a linear predictor $X\beta$
  - random errors $\epsilon$ 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 very critical as long as the sample size is not too small
- The components of 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: $\epsilon \sim N(0, \sigma^2\mathbf{I})$

# 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 $Y$
- 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 $Y$

# LME Examples: Exmaple 2

# LME Examples: Exmaple 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/BeyondTan
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(>|
## (Intercept)     0.71490545 0.01233741 57.9461618 0.000000e
## treatment_idx2 -0.07802047 0.01701121 -4.5864155 4.835037e
## treatment_idx3  0.00914741 0.01718859  0.5321791 5.946707e
## treatment_idx4  0.04971562 0.01633230  3.0440051 2.369903e
```

# 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
summary(lmer.obj)$coefficients
```

```
##                    Estimate Std. Error          df    t va
## (Intercept)     0.699786009 0.03484986    4.901964 20.0800
## treatment_idx2 -0.017490109 0.01726513 1723.485832 -1.0130
## treatment_idx3  0.009353984 0.01657856 1720.292658  0.5642
## treatment_idx4  0.029448530 0.01656107 1719.621372  1.7781
```

# 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 | $4.8 \times 10^{-6}$ | 0.595 | $2.4 \times 10^{-3}$ |
| 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



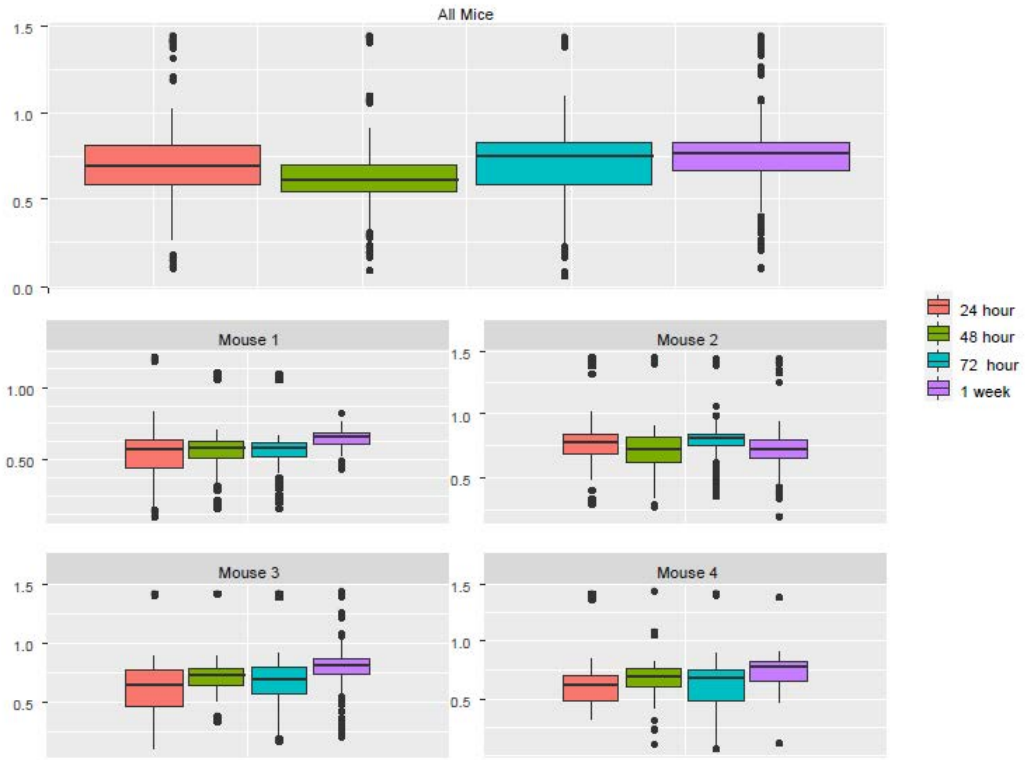


**Figure 1:** The boxplots of Ca++ event frequencies measured at four time points. (A) Boxplot of Ca++ event frequencies using the pooled neurons from four mice. (B) boxplots of Ca++ event frequencies stratified by individual mice.

# 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.
- 622 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)
Ex3=read.csv("https://www.ics.uci.edu/~zhaoxia/Data/BeyondTan
```

# Example 3: LM vs LME



```
#### wrong analysis: using the linear model
summary(lm(res~treatment, data=Ex3[!is.na(Ex3$res),])) #0.003
#### wrong anlaysis using t tests (paired or unpaired)
t.test(Ex3[Ex3$treatment==1,"res"], Ex3[Ex3$treatment==2,"res
t.test(Ex3[Ex3$treatment==1,"res"], Ex3[Ex3$treatment==2,"res
t.test(Ex3[Ex3$treatment==1,"res"], Ex3[Ex3$treatment==2,"res

#LME
lme.obj1=lme(res~ treatment, random =~1| midx/cidx, data= Ex3
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)

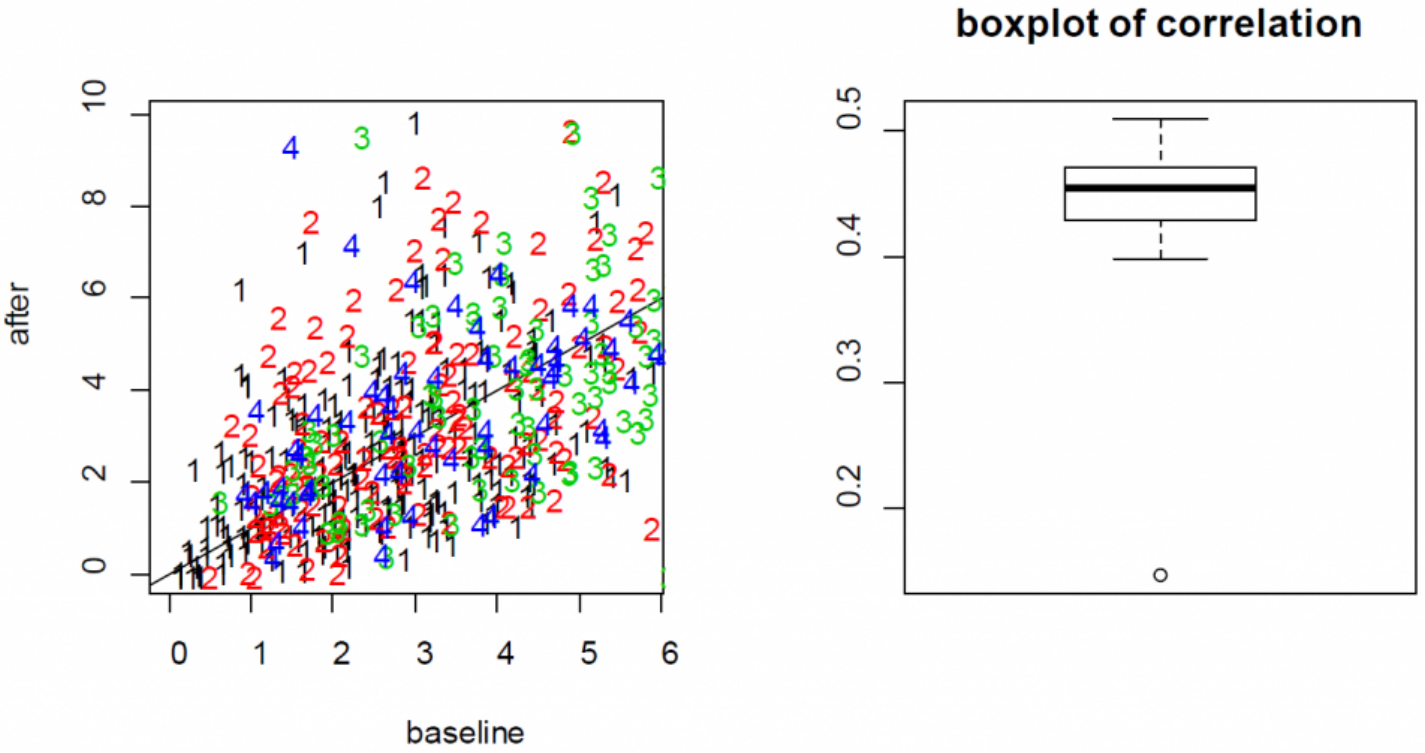


**Figure 2:** (Left) the scatter plot of Ca++ event integrated amplitude at baseline vs 24h after treatment for the neurons from four mice (labeled as 1, 2, 3 and 4) indicates that the baseline and after-treatment measures are positively correlated. (Right) boxplot of the baseline and after-treatment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

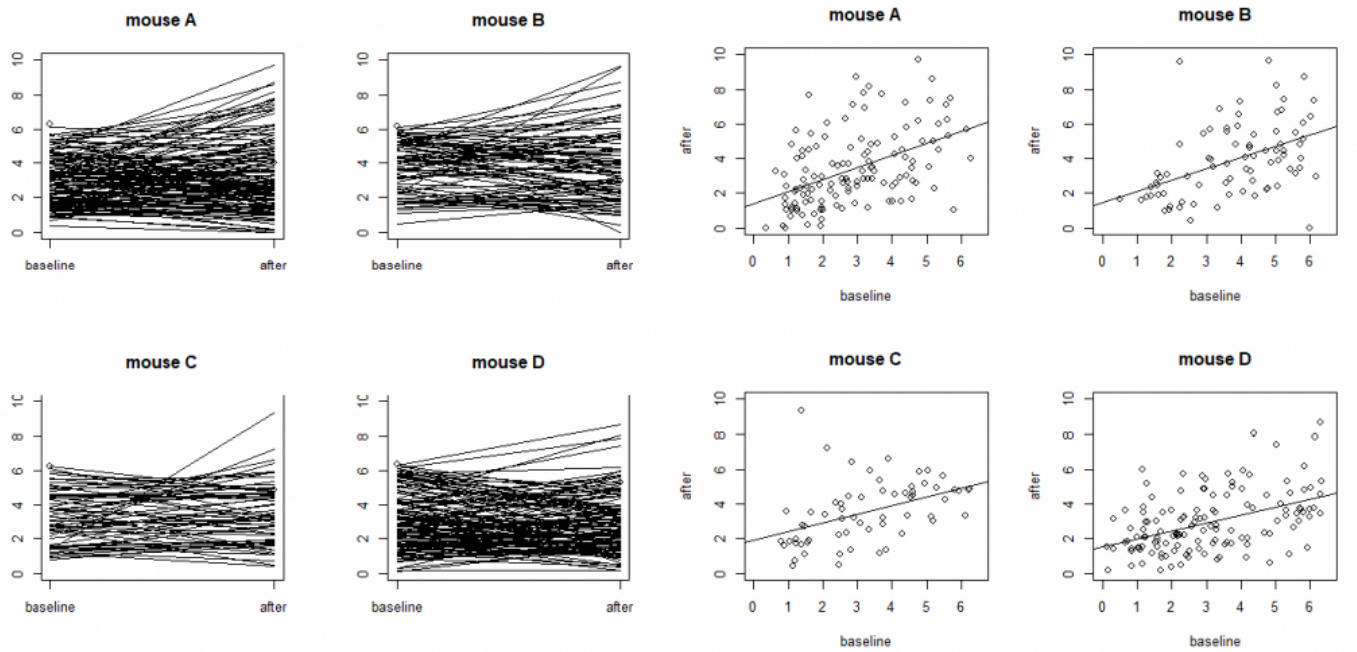


**Figure 3:** Ca++ event integrated amplitudes at baseline vs 24h after treatment for the neurons from four mice (labeled as A, B, C and D) with each dot representing a neuron. The four plots on the left are "spaghetti" plots of the four animals with each line representing the values at baseline and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

# 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 exmaple 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 much improvement

# GLMM

# 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 wront 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/B
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
anova(obj.glmm, obj.glmm.smaller)
#alternatively, one can use the "drop1" function to test the
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 equating equation (GEE)
- Oftentimes, Bayesian approaches are easier to converge