UC Irvine ISI-BUDS Day 13

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Study Goals

Study Goals

- Introduction: Revisiting LM
- Correlated Data
  - Sources of correlation
  - The consequence of ignoring data dependence: a simulation study
- Model correlated data using linear mixed-effects model
- Examples of LME: Example 1
- 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/

Introduction

Revisiting LM

- Basic assumptions of LM

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

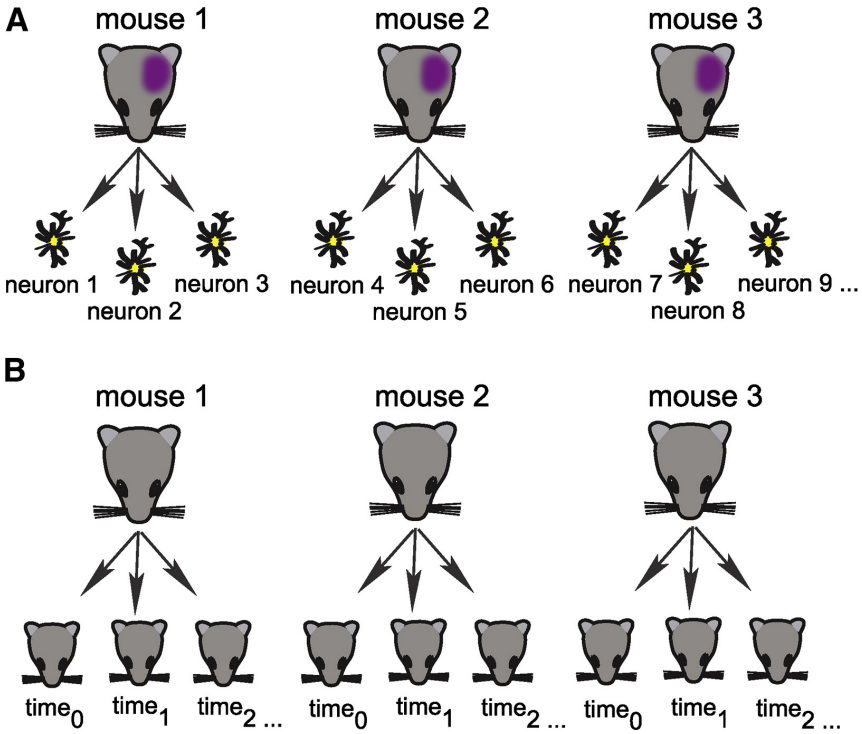
  - $E(\epsilon_i) = 0$, which is equivalent to $E(Y_i|X_i) = \beta_0 + x_{i1} \times \beta_1 + \ldots + x_{ip} \times \beta_p$
  - $Var(\epsilon_i) = \sigma^2$. Note, this is equivalent to say $Var(Y_i|X_i) = \sigma^2$.
  - $(\epsilon_1, \cdots, \epsilon_n)$ are mutually independent
- Question: what if the observations are dependent?

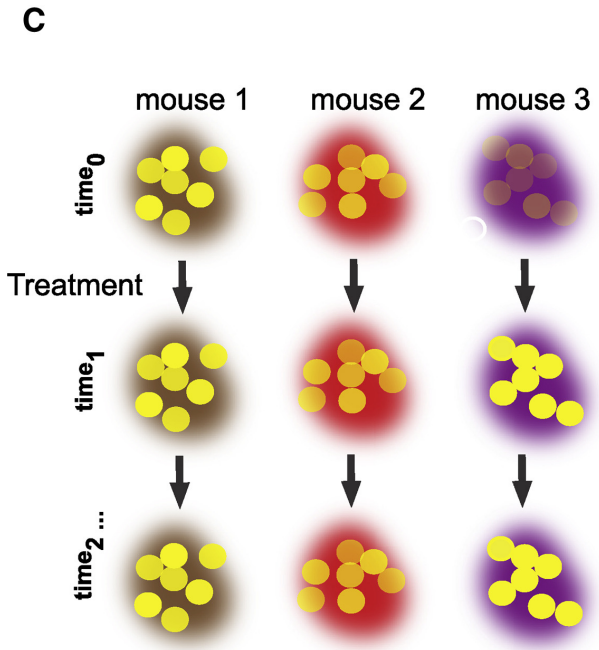
Correlated Data

Sources of correlation

- Clustered data: all fifth graders in the Irvine Unified School District
- Data with spatial correlation: today's highest temperatures of all cities in California
- Data with temporal correlation: hourly temperatures of Irvine within a day
- ...

Sources of correlation





Example 1: Data

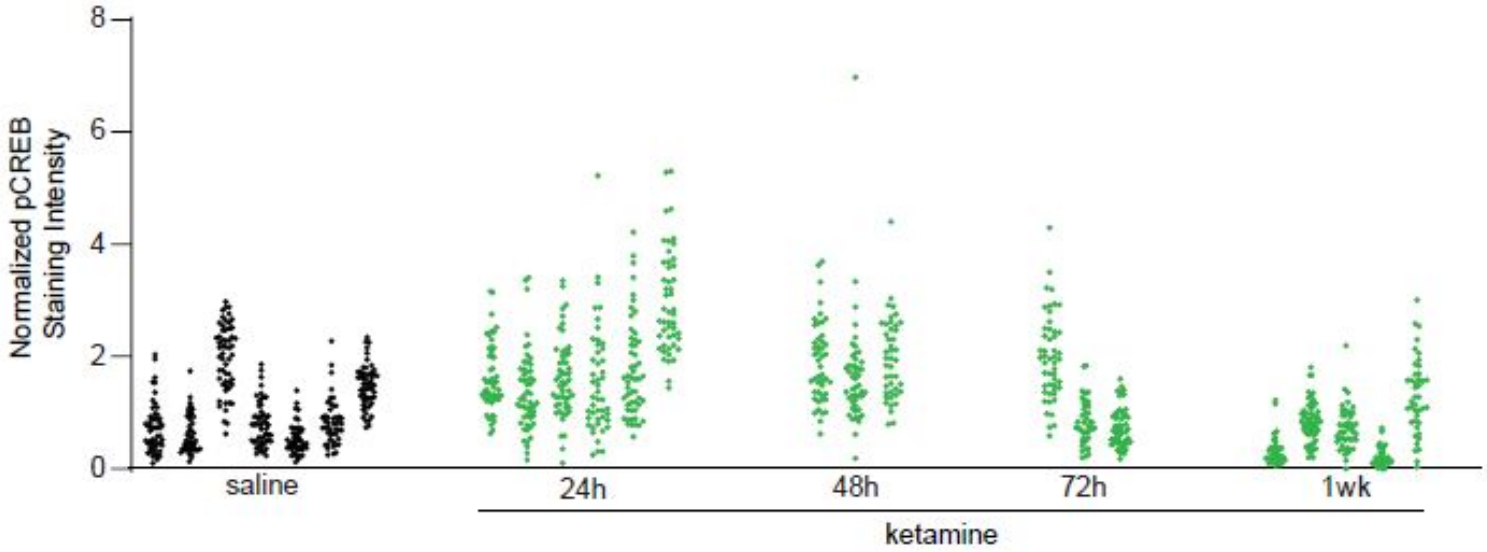


Figure 1: Normalized pCREB staining intensity from 1,200 neurons. The values in each cluster were from one animal. In total, pCREB was measuredfrom 24 mice: saline (7 mice), 24h (6 mice), 48h (3 mice), 72h (3 mice), 1week (5 mice) after treatment.

Example 1: Data


```
Ex1 = read.csv("https://www.ics.uci.edu/~zhaoxia/Data/BeyondT
#factor the treatment IDs
Ex1$treatment_idx = as.factor(Ex1$treatment_idx)
dim(Ex1)# checking the dimensions of the dataset
```

```
## [1] 1200    3
```

```
names(Ex1)# checking the names of each column
```

```
## [1] "res"           "treatment_idx" "midx"
```

Example 1: Data


```
table(Ex1$treatment_idx)
```

```
##
##   1   2   3   4   5
## 357 309 139 150 245
```

```
table(Ex1$midx)
```

```
##
##  1  2  3  4  5  6  7  8  9 10 11 12 13 14 15 16 17 18 19 2
## 53 49 56 52 46 47 54 52 54 54 47 53 49 47 48 44 50 45 55 4
```

```
#table(Ex1$treatment, Ex1$midx)
```

Example 1: Ignore data dependence (incorrect analysis)

- A common analysis is to ignore data dependence
- Representative descriptions of inappropriate analyses
  - "t(28656) = 314 with $p < 10^{-10}$ over a total of n=28657 neurons pooled across six mice,"
  - "n = 377 neurons from four mice, two-sided Wilcoxon signed rank test,"
  - "610 A cells, 987 B cells and 2584 C cells from 10 mice, oneway ANOVA and Kruskal–Wallis test,"
  - "two-sided paired t test, n=1597 neurons from 11 animals, d.f. = 1596,"

Example 1: Ignore data dependence (incorrect analysis)

```
obj.lm=lm(res~treatment_idx, data=Ex1)
coef.table=summary(obj.lm)$coefficients
row.names(coef.table)=c("Saline", "24h-S", "48h-S", "72h-S",
print(coef.table)
```

```
##              Estimate Std. Error   t value      Pr(>|t|)
## Saline  1.0261907 0.03997259 25.672363 4.064778e-116
## 24h-S   0.7828564 0.05868406 13.340189  6.040147e-38
## 48h-S   0.8135287 0.07550847 10.774006  6.760583e-26
## 72h-S   0.1605790 0.07348870  2.185084  2.907634e-02
## 1wk-S  -0.3604732 0.06265813 -5.753015  1.112796e-08
```

Example 1: Ignore data dependence (incorrect analysis)


```
anova(obj.lm)
```

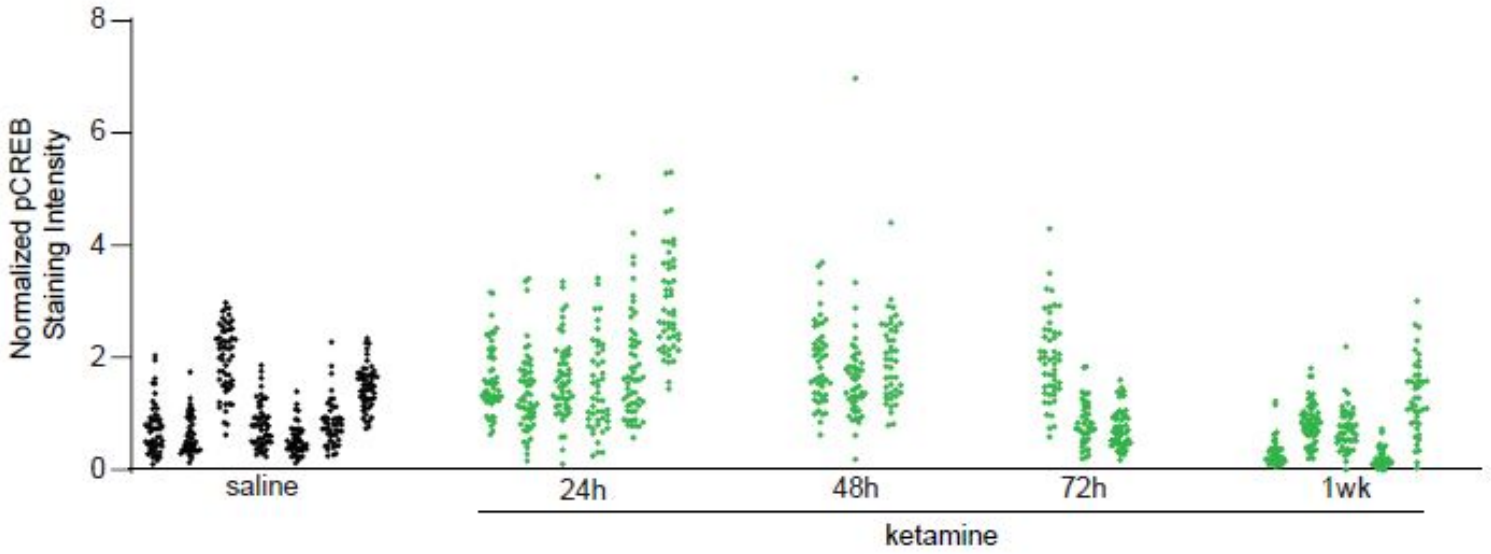
```
## Analysis of Variance Table
##
## Response: res
##                  Df Sum Sq Mean Sq F value    Pr(>F)
## treatment_idx     4 246.62  61.656  108.09 < 2.2e-16 ***
## Residuals      1195 681.65   0.570
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 '
```

```
#The same analysis can be done by the "aov" function
```

- What is wrong with the analysis?

Example 1: Understand the dependence in data

- From Figure 1, what can we say about observations within and between animals?
- Data are clustered in animals (mice)



Intra-class Correlation (ICC)

- We use ICC to quantify the dependence due to clustering. It is defined as

$$ICC = \frac{\sigma_b^2}{\sigma_b^2 + \sigma_e^2},$$

where

- σ_b^2 denotes the between-class variance
- σ_e^2 denotes the within-class variance
- The ICC for naturally occuring clusters is often between 0 and 1
- $ICC = 0$: the data are uncorrelated
- $ICC = 1$: all the observations in each cluster are identical

Example 1: Intra-class Correlation (ICC)


```
### load the ICC library
library(ICC)
### conduct ICC analysis by organizing all the information in
icc.analysis=data.frame(n=rep(0,5), icc=rep(0,5), design.effe
effective.n=rep(0,5), M=rep(0,5), cells=rep(0,5))
# The ICC is computed as follows (code won't show in slides)
```

Intra-class Correlation (ICC): Example 1

Table 1. ICC, design effect, and effective sample size for the 5 groups in example 1

	Saline (7 mice)	24 h (6 mice)	48 h (3 mice)	72 h (3 mice)	1 week (5 mice)
No. cells	357	209	139	150	245
ICC	0.61	0.33	0.02	0.63	0.54
Design effect	32.0	17.7	1.8	31.8	26.8
Effective sample size	11.1	17.5	76.9	4.7	9.1

ICC and the design effect were the lowest at 48 h, when the data were relatively homogeneous across animals. At baseline and 72 h, the data were noticeably heterogeneous across animals, leading to high ICC.

Intra-class Correlation (ICC): Example 1

- The dependency due to clustering is substantial
- the 1,200 neurons should not be treated as 1,200 independent cells
- Design effect: $D_{eff} = 1 + (M - 1)ICC$, where M is the average cluster size
- Effective sample size: $n_{eff} = n/D_{eff}$.

The consequence of ignoring data dependence: a simulation study

- We generated 1000 data sets
  - each follows the same ICC structure of Example 1
  - data are simulated under the assumptino of no difference between the five treatments/conditions
  - each data set is analyzed with the regular LM/ANOVA, without accounting for the data dependence.
  - significance level is set at $\alpha = 0.05$.
- Let's guess: What is the type I error rate (proportion of times rejecting the null hypothesis wrongly)?

Type I vs Type II erros

- A Type I error (false positive): rejecting the null hypothesis when it is true
- A Type II error (false negative): failing to reject the null hypothesis when it is false
- Significance level α:
- If the null hypothesis is true and $\alpha = 0.05$, the type I error rate of a valid test should be 0.05

The consequence of ignoring data dependence: a simulation study


```
source("https://www.ics.uci.edu/~zhaoxia/Data/BeyondTandANOVA
```

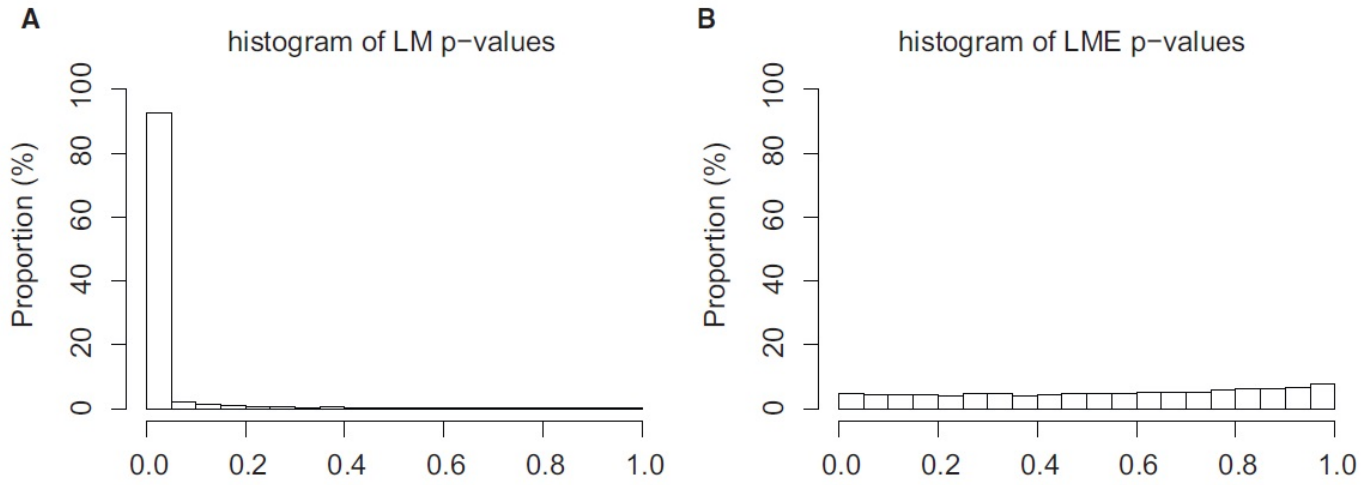


Figure 3. Histograms of p values using simulated data that assume (1) no treatment effects and (2) the same sample sizes and correlation structure with example 1
(A) Histogram of the p values from the inappropriate method (linear model) shows that ignoring the correlation structure of the data led to a surprisingly high type I error rate (90%) at significance level $\alpha = 0.05$.
(B) Histogram of the p values from LME.

Linear-Mixed Effects Model

A Motivating Example of LME: Example 1 (the wrong model)

- The model without accounting for dependence:

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

where

  - n_i is the number of observations from the ith mouse and $\sum_{i=1}^{24} n_i = 1200$
  - ϵ_{ij} represents the deviation in pCREB immunoreactivity of observation (cell) j in mouse i from the mean pCREB immunoreactivity of mouse i. We assume that the errors ϵ_{ij}'s are i.i.d. from $N(0, \sigma^2)$.

A Motivating Example of LME: Example 1 (the wrong model)

- The model without accounting for dependence:

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

where the coefficients $\beta_0, \beta_1, \beta_2, \beta_3, \beta_4$ are assumed to be fixed but unknown parameters

  - β_0 is the intercept (the mean of the baseline)
  - β_1 is the effect (change) at 24h, compared to the baseline
  - β_2 is the effect (change) at 48h, ...
  - β_3 is the effect (change) at 72h, ...
  - β_4 is the effect (change) at 1wk, ...

A Motivating Example of LME: Example 1 (the wrong model)

- The model without accounting for dependence:

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

- We use four dummy variables to denote treatments/conditions, which are the time after treatment
  - $x_{ij,1} = 1$ for 24 hours and 0 otherwise
  - $x_{ij,2} = 1$ for 48 hours and 0 otherwise
  - $x_{ij,3} = 1$ for 72 hours and 0 otherwise
  - $x_{ij,4} = 1$ for 1 week and 0 otherwise after ketamine treatments, respectively.

A Motivating Example of LME: Example 1

- Cells from the same animal share the same environment

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

- We add the subject-specific effect u_i into the LM
  - $(u_1, \cdots, u_{24})$ are assumed to be independent and identically distributed (i.i.d.) from $N(0, \sigma_b^2)$
  - In addition, $(u_1, \cdots, u_{24})$ are assumed to be independent from the random errors ϵ_{ij}'s.

A Motivating Example of LME: Example 1

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

* We call this model a mixed-effects model because there are two types of coefficeints + $(u_1, \cdots, u_{24})$ are random-effect coefficients + $(\beta_0, \cdots, \beta_4)$ are fixed-effect coefficients

A Motivating Example of LME: Example 1

- Including the random coefficients introduces correlation between two observations from the same cluster:
  - for $j \neq j'$, $cov(Y_{ij}, Y_{ij'}) = ... = \sigma_b^2$
  - for $i \neq i'$, $cov(Y_{ij}, Y_{i'j'}) = 0$

A Better Representation of the model

- Introduce dummy variables for the animals: let the vector $(z_{ij,1}, \ldots, z_{ij,24})$ be the dummy variables for the cluster/animal memberships such that $z_{ij,k} = 1$ for $i = k$ and 0 otherwise.
- Then the LME can be rewritten to

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

$$i = 1, \ldots, 24; j = 1, \ldots, n_i;$$

LME

- A more compact form of the model presented in the previous slide:

$$Y = \mathbf{1}\beta_0 + X\beta + Zu + \epsilon$$

- Remark 1: Example 1 includes treatment effects as fixed effects. Similar to LM and GLM, when available and sensible, other covariates should be added

LME: fixed vs random effects

- Remark 2: You might wonder why do we treat u_i's as random, rather than fixed effects.
- Fixed-effects: typically, a fixed effect captures a parameter at the population level
- Random-effects:
  - A random effect captures cluster-specific effects (e.g., due to cells clustered in animals)
  - In many situations, the number of clusters (e.g., patients in a longitudinal study) is large.There would be too many parameters to estimate if we treat u_i's as fixed.
  - Subject-specific effects are typically of no direct scientific interest
- Remark 3: Other random effects might also be necessary. e.g., we will discuss random slopes in an example

Fit an LME model

- The parameters need to be estimated:

$$\beta_0, \beta_1, \cdots, \beta_p, \sigma^2, \sigma_b^2$$

- Two methods to estimated parameters
  - Maximum likelihood estimation
  - REML: restricted/residual maximum likelihood estimates
  - Two main packages:
    - nlme: lme
    - lme4: lmer (doesn't provide p-value) and glmm

Fit an LME model: relevant packages

Table 5. Selected R packages and functions for mixed-effects modeling and statistical inference

Package name	Functions related to mixed-effect modeling
nlme	*lme*: fit a linear mixed-effects model
lme4	*lmer*: fit a linear mixed-effects model
	glmm: fit a generalized linear mixed-effects model
brms	It can conduct Bayesian mixed-effects modeling
lmerTest	It can perform hypothesis testing on fixed and random effects based on models from lme4::lmer
emmeans	It can provide adjusted p values for pairwise and treatments versus control comparisons
pbkrtest	It can perform the F-test (Kenward-Roger and Satterthwaite type) and parametric bootstrap test
car	car::Anova provides large-ample Wald test or F-test with Kenward-Roger denominator degrees of freedom
sjPlot	It can provide visualization and create manuscript-style tables

LME Examples: Example 1

Example 1: use nlme::lme

```
################### Linear Mixed-effects Model ###############
#use nlme::lme
library(nlme) #load the nlme library
# The nlme:lme function specifies the fixed effects in the fo
# (first argument) of the function, and the random effects
# as an optional argument (random=). The vertical bar | denot
# the cluster is done through the animal id (midx)
obj.lme=lme(res~treatment_idx, data= Ex1, random = ~ 1|midx)
#summary(obj.lme)
```

Example 1: evaluate the overall significance of a factor with nlme::lme


```
#Wald F-test from an lme object
obj.lme=lme(res~treatment_idx, data= Ex1, random = ~ 1|midx)
anova(obj.lme) #Wald F-tes
```

```
##                numDF denDF  F-value p-value
## (Intercept)        1  1176 142.8589  <.0001
## treatment_idx      4    19   4.6878  0.0084
```

Example 1: evaluate the overall significance of a factor with nlme::lme


```
#Likelihood ratio test from lme objects
# notice the argument of the option "method"
# which calls for using ML instead of REML
obj.lme0.ml=lme(res~1, data= Ex1, random = ~ 1|midx, method="
obj.lme.ml=lme(res~treatment_idx, data= Ex1, random = ~ 1|mid
anova(obj.lme0.ml, obj.lme.ml)
```

```
##                Model df       AIC       BIC    logLik   Test  L
## obj.lme0.ml        1  3 2281.441 2296.712 -1137.721
## obj.lme.ml         2  7 2272.961 2308.592 -1129.481 1 vs 2 16
```

Example 1: evaluate the overall significance of a factor with nlme::lme


```
#equivalently, one can conduct LRT using drop1
drop1(obj.lme.ml, test="Chisq")
```

```
## Single term deletions
##
## Model:
## res ~ treatment_idx
##                Df    AIC   LRT Pr(>Chi)
## <none>            2273.0
## treatment_idx   4 2281.4 16.48 0.002438 **
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 '
```

Example 1: Compare LM and LME

- Compare LM and LME

Table 2. p values for comparing pCREB immunoreactivity at each time point (24 h, 48 h, 72 h, and 1 week) after ketamine treatment to the baseline (saline)

	Overall	24 h	48 h	72 h	1 week
Linear model (ANOVA)	1.2×10^{-78}	6.0×10^{-38}	6.8×10^{-26}	0.0291	1.1×10^{-8}
LME	0.0029	0.0049	0.0164	0.5601	0.2525

The "Overall" column corresponds to the null hypothesis of no difference among the 5 groups (example 1). The LME p values are based upon the *lme* function in the *nlme* package, in which the denominator degrees of freedom are determined by the animal grouping level (Pinheiro et al., 2007). The methods for obtaining more accurate p values with adjustments for multiple comparisons can be found in the supplemental information.

Example 1: use lme4::lmer

```
library(lme4) #load the lme4 library
obj.lmer=lmer(res ~ treatment_idx+(1|midx), data=Ex1)
```

Example 1: use lme4::lmer


```
library(lmerTest)
```

```
## Warning: package 'lmerTest' was built under R version 4.1.
```

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

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

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

```
obj.lmer=lmerTest::lmer(res ~ treatment_idx+(1|midx), data=Ex
#when ddf is not specified, the F test with Satterthwaite's m
```

Example 1: use lme4::lmer

```
summary(obj.lmer, ddf="Kenward-Roger")
```

```
## Linear mixed model fit by REML. t-tests use Kenward-Roger'
## lmerModLmerTest]
## Formula: res ~ treatment_idx + (1 | midx)
##    Data: Ex1
##
## REML criterion at convergence: 2264.5
##
## Scaled residuals:
##     Min      1Q  Median      3Q     Max
## -2.5388 -0.5761 -0.1129  0.4721  8.8601
##
## Random effects:
##  Groups   Name        Variance Std.Dev.
```

Example 1: use lme4::lmerr

```
#likelihood ratio test
obj.lmer.ml=lme4::lmer(res ~ treatment_idx+(1|midx), data=Ex1
obj.lmer0.ml=lme4::lmer(res ~ 1+(1|midx), data=Ex1, REML=F)
anova(obj.lmer0.ml, obj.lmer.ml)
```

```
## Data: Ex1
## Models:
## obj.lmer0.ml: res ~ 1 + (1 | midx)
## obj.lmer.ml: res ~ treatment_idx + (1 | midx)
##              npar    AIC    BIC  logLik deviance  Chisq Df
## obj.lmer0.ml    3 2281.4 2296.7 -1137.7   2275.4
## obj.lmer.ml     7 2273.0 2308.6 -1129.5   2259.0 16.48  4
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 '
```

Adjustment for multiple comparisons

Adjustment for multiple comparisons

- What is the issue of multiple comparisons
- Suppose the null hypothesis is true. What is the type I error rate if we conduct a test with $\alpha = 0.05$?
- Suppose M null hypotheses are true. What is the probability of making at least one mistake if we use $\alpha = 0.05$ for each test?
  - This probability is called the family wise error rate
- Mathematical derivation or simulation will show that the family wise error rate is much greater than 0.05
- Procedures have been developed to control for the family wise error rate and other types error rate (e.g., false discovery rate)

Adjustment for multiple comparisons


```
##  contrast estimate    SE   df t.ratio p.value
##  1 - 2     -0.8194 0.289 19.0  -2.835  0.0704
##  1 - 3     -0.8429 0.359 19.1  -2.349  0.1727
##  1 - 4     -0.1898 0.359 19.0  -0.529  0.9832
##  1 - 5      0.3200 0.304 19.0   1.051  0.8283
##  2 - 3     -0.0235 0.368 19.0  -0.064  1.0000
##  2 - 4      0.6296 0.367 19.0   1.713  0.4496
##  2 - 5      1.1394 0.315 19.0   3.621  0.0138
##  3 - 4      0.6531 0.425 19.0   1.538  0.5517
##  3 - 5      1.1629 0.380 19.1   3.062  0.0447
##  4 - 5      0.5098 0.380 19.0   1.343  0.6690
##
## Degrees-of-freedom method: kenward-roger
## P value adjustment: tukey method for comparing a family of
```

Adjustment for multiple comparisons


```
# the default method of degrees of freedom is Kenward-Roger's
contrast(emmeans(obj.lmer, specs="treatment_idx"), "trt.vs.ct
```

```
##  contrast estimate    SE   df t.ratio p.value
##  2 - 1       0.819 0.289 19.0   2.835  0.0364
##  3 - 1       0.843 0.359 19.1   2.349  0.0965
##  4 - 1       0.190 0.359 19.0   0.529  0.9219
##  5 - 1      -0.320 0.304 19.0  -1.051  0.6613
##
## Degrees-of-freedom method: kenward-roger
## P value adjustment: dunnettx method for 4 tests
```