FMRI Analysis

Experiment Design

Scanning

Pre-Processing

Individual Subject Analysis

Group Analysis

Post-Processing

Overview

- Why do we need to do group analysis?
- Fixed-effects analysis
- Mixed-effects analysis
  - Nonparametric approach
    - **3dWilcoxon, 3dMannWhitney**, **3dKruskalWallis**, **3dFriedman**
  - Parametric approach
- Traditional parametric analysis
  - Use regression coefficients (β) only
  - 3dttest, 3dANOVA/2/3, 3dRegAna, GroupAna, 3dLME
- New group analysis method
  - Use both β and *t*-statistic: mixed-effects meta analysis (MEMA)
  - 3dMEMA

• Group Analysis: Fixed-Effects Analysis

- Number of subjects $n < 6$
- Case study: can't generalize to whole population
- Simple approach (**3dcalc**)
  - $t = \sum t_{ii} / \sqrt{n}$
- Sophisticated approach
  - Fixed-effects meta analysis (**3dcalc**)
    - $\beta = \sum(b_i/\sqrt{v_i}) / \sum(1/\sqrt{v_i})$
    - $t = \beta \sum(1/\sqrt{v_i}) / \sqrt{n}$, v_i = variance for i-th regressor
  - Direct fixed-effects analysis (**3dDeconvolve/3dREMLfit**)
    - Combine individual data and then run regression

• Group Analysis: Non-Parametric Analysis

- 4 < number of subjects < 10
- No assumption of normality; statistics based on ranking
- Programs
  - **3dWilcoxon** (~ paired *t*-test)
  - **3dMannWhitney** (~ two-sample *t*-test)
  - **3dKruskalWallis** (~ between-subjects with **3dANOVA**)
  - **3dFriedman** (~one-way within-subject with **3dANOVA2**)
- Multiple testing correction with FDR (**3dFDR**)
- Less sensitive to outliers (more robust)
- Less flexible than parametric tests
- Can't handle complicated designs with more than one fixed factor

• Group Analysis: Basic concepts in parametric approach

- Fixed factor
  - Treated as a fixed variable in the model
    - Categorization of experiment conditions (modality: visual/audial)
    - Group of subjects (gender, normal/patients)
  - All levels of the factor are of interest and included for all replications
  - Fixed in the sense inferences
    - apply only to the specific levels of the factor
    - don't extend to other potential levels that might have been included
- Random factor
  - Exclusively subject in FMRI
  - Treated as a random variable in the model
    - average + effects uniquely attributable to each subject: $N(\mu, \sigma^2)$
  - Each individual subject is of NO interest
  - Random in the sense
    - subjects serve as a random sample of a population
    - inferences can be generalized to a hypothetical population

• Group Analysis: Mixed-Effects Analysis

Parametric approach

- Number of subjects $n > 10$
- Random effects of subjects: Gaussian distribution
- Individual and group analyses: separate
- Within-subject variation ignored
- Main focus of this talk

Programs

- **3dttest** (one-sample, two-sample and paired *t*)
- **3dANOVA** (one-way between-subject)
- **3dANOVA2** (one-way within-subject, 2-way between-subjects)
- **3dANOVA3** (2-way within-subject and mixed, 3-way between-subjects)
- **3dRegAna** (regression/correlation, simple unbalanced ANOVA, simple ANCOVA)
- **GroupAna** (Matlab package for up to 5-way ANOVA)
- **3dLME** (R package for all sorts of group analysis)
- **3dMEMA** (R package for meta analysis)

• <u>Group Analysis</u> : `3dttest`

- Basic usage
  - One-sample t
    - <u>One group</u>: simple effect
    - Example: 15 subjects under condition A with H_0: $\mu_A = 0$
  - Two-sample t
    - <u>Two groups</u>: Compare one group with another
    - ~ 1-way between-subject (**`3dANOVA2 -type 1`**)
    - Unequal sample sizes allowed
    - Assumption of equal variance
    - Example: 15 subjects under A and 13 other subjects under B - H_0: $\mu_A = \mu_B$
  - Paired t
    - <u>Two conditions of one group</u>: Compare one condition with another_
    - ~ one-way within-subject (**`3dANOVA2 -type 3`**)
    - ~ one-sample t on individual contrasts
    - Example: Difference between conditions A and B for 15 subjects with H_0: $\mu_A = \mu_B$
- Output: 2 values (% and t)
- Versatile program: Most tests can be done with **`3dttest`** - piecemeal vs. bundled

- **Group Analysis: `3dANOVA`**
  - Generalization of two-sample *t*-test
    - One-way between-subject: 2 or more groups of subjects
    - H_0: no difference across all levels (groups)
    - Examples of groups: gender, age, genotype, disease, *etc.*
    - Unequal sample sizes allowed
  - Assumptions
    - Normally distributed with equal variances across groups
  - Results: 2 values (% and *t*)
  - **`3dANOVA`** vs. **`3dttest`**
    - Equivalent with 2 levels (groups)
    - More than 2 levels (groups): Can run multiple two-sample *t*-test

- **Group Analysis**: **`3dANOVA2`**
  - Designs
    - One-way within-subject (**`type 3`**)
      - Major usage
      - Compare conditions in one group
      - Extension and equivalence of paired *t*
    - Two-way between-subjects (**`type 1`**)
      - 1 condition, 2 classifications of subjects
      - Extension and equivalence two-sample *t*
      - Unbalanced designs disallowed: Equal number of subjects across groups
  - Output
    - Main effect (**`-fa`**): *F*
    - Interaction for two-way between-subjects (**`-fab`**): *F*
    - Contrast testing
      - Simple effect (**`-amean`**)
      - 1st level (**`-acontr, -adiff`**): among factor levels
      - 2nd level (interaction) for two-way between-subjects
      - 2 values per contrast: % and *t*

• Group Analysis: `3dANOVA3`

- Designs
  - Three-way between-subjects (**`type 1`**)
    - 3 categorizations of groups
  - Two-way within-subject (**`type 4`**): Crossed design AXBXC
    - Generalization of paired *t*-test
    - One group of subjects
    - Two categorizations of conditions: A and B
  - Two-way mixed (**`type 5`**): Nested design BXC(A)
    - Two or more groups of subjects (Factor A): subject classification, e.g., gender
    - One category of condition (Factor B)
    - Nesting: balanced
- Output
  - Main effect (**`-fa and -fb`**) and interaction (**`-fab`**): *F*
  - Contrast testing
    - 1st level: **`-amean, -adiff, -acontr, -bmean, -bdiff, -bcontr`**
    - 2nd level: **`-abmean, -aBdiff, -aBcontr, -Abdiff, -Abcontr`**
    - 2 values per contrast : % and *t*

• Group Analysis: Example

```
3dANOVA3 -type 4  -alevels 3  -blevels 3  -clevels 16 \
-dset 1 1 1 stats.sb04.beta+tlrc'[0]' \
-dset 1 2 1 stats.sb04.beta+tlrc'[1]' \
-dset 1 3 1 stats.sb04.beta+tlrc'[2]' \
-dset 2 1 1 stats.sb04.beta+tlrc'[4]' \
…
-fa  Category \
-fb  Affect \
-fab CatXAff \
-amean     1     T     \ (coding with indices)
-acontr    1 0 -1 TvsF \ (coding with coefficients)
-bcontr    0.5 0.5 -1 non-neu \ (coefficients)
-aBcontr   1 -1 0 : 1 TvsE-pos \ (coefficients)
-Abcontr   2 : 1 -1 0 HMvsHP \ (coefficients)

-bucket anova33
```

Model type, Factor levels

Input for each cell in ANOVA table: totally 3X3X16 = 154

F tests: Main effects & interaction

t tests: 1st order Contrasts

t tests: 2nd order Contrasts

Output: bundled

- **Group Analysis**: GroupAna
  - Multi-way ANOVA
    - Matlab script package for up to 5-way ANOVA
    - Requires Matlab plus Statistics Toolbox
    - GLM approach (slow)
    - Powerful: Test for interactions
    - Downside
      - Difficult to test and interpret simple effects/contrasts
      - Complicated design, and compromised power
    - Heavy duty computation: minutes to hours
      - Input with lower resolution recommended
      - Resample with **`adwarp -dxyz #`** and **`3dresample`**
    - Can handle both volume and surface data
    - Can handle following unbalanced designs (two-sample *t* type):
      - 3-way ANOVA type 3: BXC(A)
      - 4-way ANOVA type 3: BXCXD(A)
      - 4-way ANOVA type 4: CXD(AXB)
  - See http://afni.nimh.nih.gov/sscc/gangc for more info

• Group Analysis: 3dLME

An R package

- Open source platform
- Linear mixed-effects (LME) modeling
- Versatile: handles almost all situations in one package
  - Unbalanced designs (unequal number of subjects, missing data, etc.)
  - ANOVA and ANCOVA, but unlimited factors and covariates
  - Able to handle HRF modeling with basis functions
  - Violation of sphericity: heteroscedasticity, variance-covariance structure
  - Model fine-tuning
- No scripting
- Disadvantages
  - High computation cost (lots of repetitive calculation)
  - Sometimes difficult to compare with traditional ANOVA
- Still under development
- See http://afni.nimh.nih.gov/sscc/gangc/lme.html for more information

• Group Analysis: 3dLME

- Running LME: A more complicated example (still testing)
  - HRF modeled with 6 tents
  - Null hypothesis: no HRF difference between two conditions

```
Data:Volume                                <-- either Volume or Surface
Output:test                                <-- any string (no suffix needed)
MASK:Mask+tlrc.BRIK                        <-- mask dataset
FixEff:Time-1                              <-- model formula for fixed effects
COV:                                       <-- covariate list
RanEff:TRUE                                <-- random effect specification
VarStr:weights=varIdent(form=~1|Time)      <-- heteroscedasticity?
CorStr:correlation=corAR1(form=~Order|Subj) <-- correlation structure
SS: sequential                             <-- sequential or marginal
Subj      Time    TimeOrder   InputFile
Jim       t1         1    contrastT1+tlrc.BRIK
Jim       t2         2    contrastT2+tlrc.BRIK
Jim       t3         3    contrast3+tlrc.BRIK
Jim       t4         4    contrast4+tlrc.BRIK
......
```

• Group Analysis: 3dLME

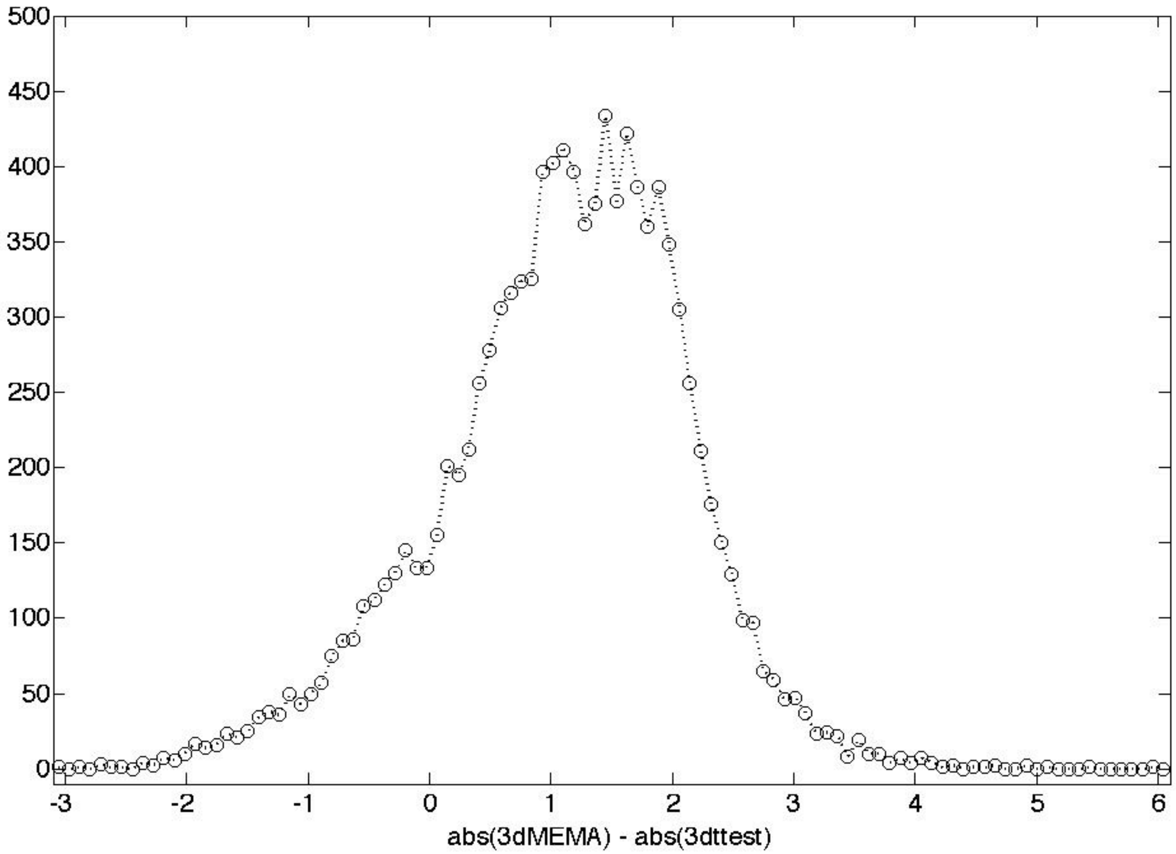
- Running LME: model fine-tuning (planning)
  - How to specify 4 structures:

```
FixEff:Time-1                          <-- model formula for fixed effects
RanEff:TRUE                            <-- random effect specification
VarStr:weights=varIdent(form=~1|Time)  <-- heteroscedasticity?
CorStr:correlation=corAR1(form=~Order|Subj) <-- correlation
```

  - Pick up a most interesting voxel
  - Start with a reasonably simple model, and compare alternatives
    - Add or reduce fixed and random effects
    - Vary variance and correlation structures
  - Problems
    - The best model at one voxel might not be true for other voxels
    - More sophisticated model means more parameters and longer running time
    - Solution: ROI analysis – analyze each ROI separately!

Appetizers for the new approach

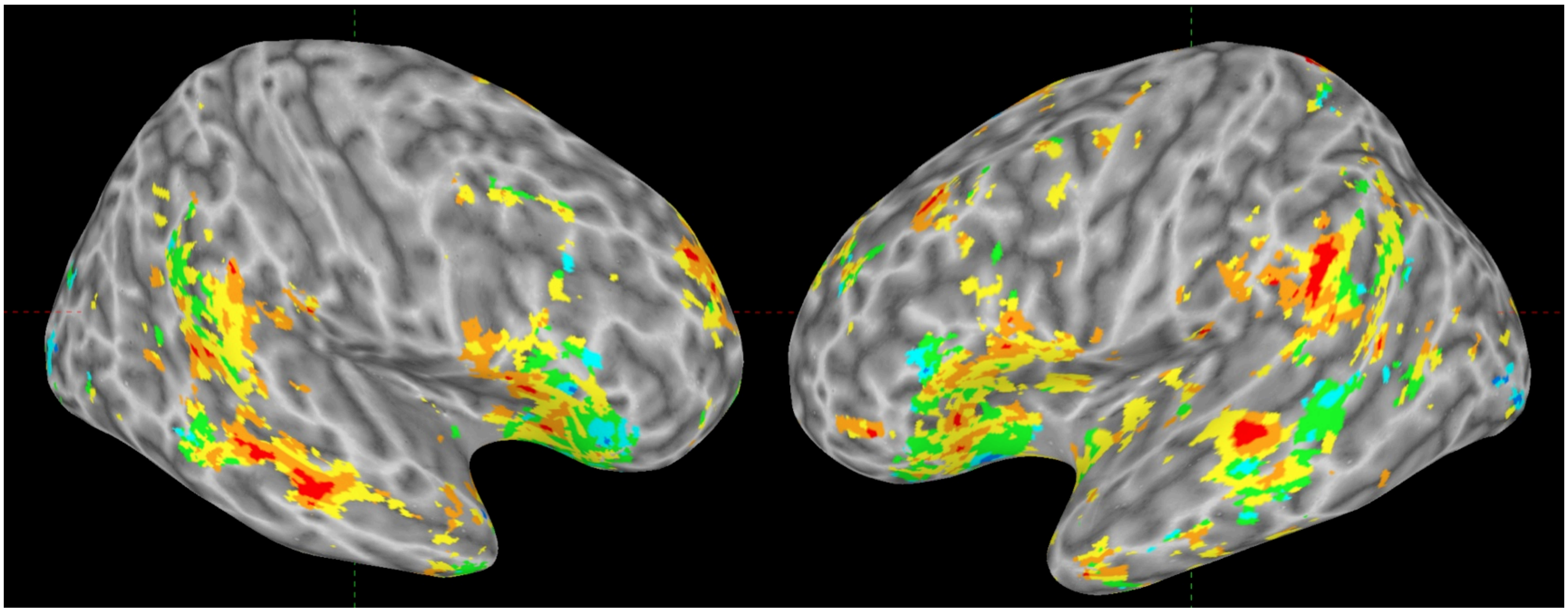
- 3dMEMA performance (vs. conventional approaches)



Majority of significant voxels with 3dMEMA gained power with a threshold of 2.0 for *t* (30). Courtesy of Vincent Costa, Univ. of Florida

Appetizers

- 3dMEMA performance (vs. conventional approaches)



Majority of significant voxels with 3dMEMA gained power (red >= 2.8, 1.7 <= orange < 2.8; 0.5 <= yellow < 1.7; -0.5 <= green < 0.5; blue <= -0.5) with a threshold of 2.0 for *t*(30). Courtesy of Vincent Costa, Univ. of Florida

Why new group analysis approach?

- Our ultimate goal is not just to gain statistical power
- Old group analysis approach
  - Take $\boldsymbol{\beta}$'s from each subject, and run *t*-test, AN(C)OVA, LME
  - Two assumptions
    - A: Within/intra-subject variability (standard error, sampling error) is relatively small compared to cross/between/inter-subjects variability
    - B: Within/intra-subject variability roughly the same across subjects
  - Violations seem everywhere: violating either can lead to suboptimal/invalid analysis
    - Common to see 40% up to 100% variability due to within-subject variability
    - Cross-subject variability in sampling error (within/intra-subject variability)

How can we do it differently?

- For each effect estimate (β or linear combination of β's)
  - Information regarding our confidence about the effect?
    - Reliability/precision/efficiency/certainty/confidence: standard error (SE)!
    - SE of an effect = estimated standard deviation (SD) of the effect
    - Smaller SE ➔ higher reliability/precision/efficiency/certainty/confidence
  - t-statistic of the effect
    - Signal-to-noise or effect vs. uncertainty: $t = \beta/\mathrm{SE}$
    - SE contained in t-statistic: $\mathrm{SE} = \beta/t$
  - Trust those β's with high reliability/precision (small SE) through weighting/compromise
    - β estimate with high precision (lower SE) has more say in the final result
    - β estimate with high uncertainty gets downgraded

Differentiate effects based on precision

- Dealing with outliers
  - Unreliable estimate (small t): small/big β + big SE
    - Will automatically be downgraded
    - May still slightly bias cross-subjects variability estimate to some extent, leading to unfavorable significance testing, but much better than conventional approach
  - Reliable estimate (big t): small/big β + small SE
    - Weighting only helps to some extent: if one subject has extremely small SE (big t), the group effect may be dominated by this subject
    - Needs delicate solutions: fundamentally why outliers?
      - Brain level: Considering ovariate(s)? Grouping subjects?
      - Singular voxels: special modeling on cross-subject variance

Running 3dMEMA

- Currently available analysis types (+ covariates allowed)
  - One-sample: one condition with one group
  - Two-sample: one condition across 2 groups with homoskedasticity (same variability)
  - Paired-sample: two conditions with one group
  - Two-sample: one condition across 2 groups with heteroskedasticity (different variability)
- Output
  - Group level: % signal change + Z/t-statistic, τ^2 + Q
  - Individual level: λ + Z for each subject
- Mode
  - Sequential mode on terminal
  - Batch mode: `R CMD BATCH cmds.R diary.txt &`
  - Remote running: `nohup R  CMD BATCH cmds.R diary.txt &`

3dMEMA limitations

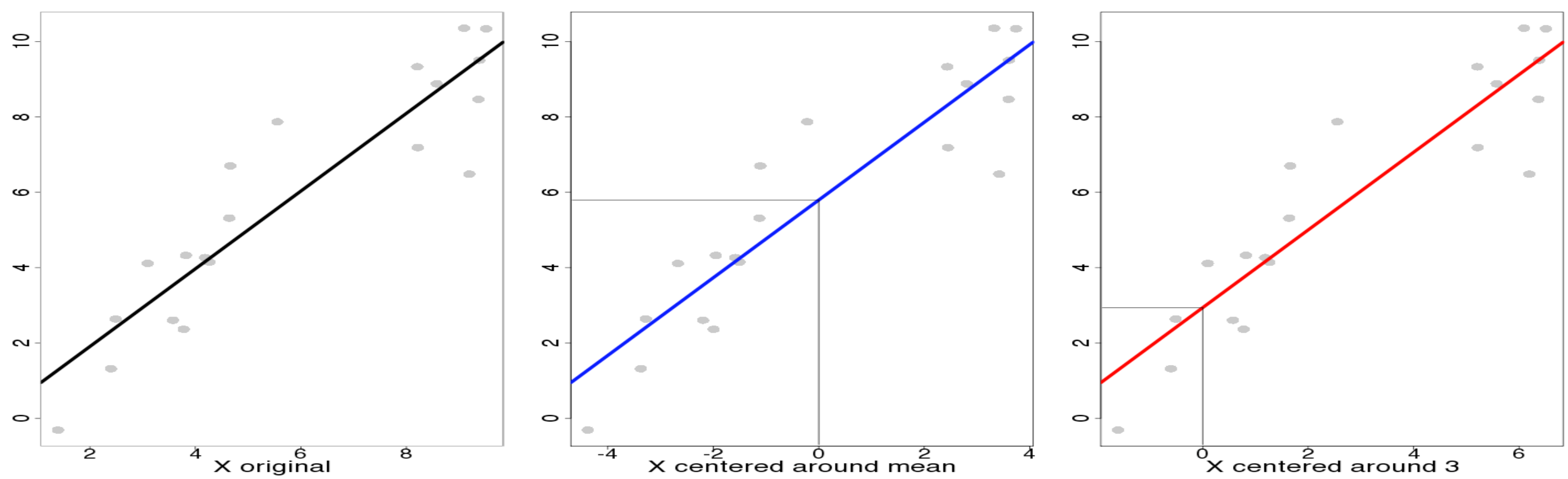
- Basis functions?
  - Stick with 3dLME for now
- ANOVA?
  - Extension difficult
  - *t*-tests should be no problem
  - *F*-tests?
    - Some of them boil down to *t*-tests, for example: *F*-test for interaction between A and B (both with 2 levels) with "3dANOVA3 -type 5…": equivalent to *t*-test for (A1B1-A1B2)-(A2B1-A2B2) or (A1B1-A2B1)-(A1B2-A2B2), but we can say more with *t* than *F*: a positive *t* shows A1B1-A1B2 > A2B1-A2B2 and A1B1-A2B1 > A1B2-A2B2
    - Do something for other *F* in the future?

Covariates

- Covariates
  - May or may not be of direct interest
  - Confounding, nuisance, or interacting variables
  - Subject-level
  - Controlling for variability in the covariate
  - Continuous or discrete?
  - One-sample model $y_i = \alpha_0+\alpha_1 x_i+ \delta_i + \varepsilon_i$, for ith subject
  - Two-sample model $y_i = \alpha_0+\alpha_1 x_{1i}+\alpha_2 x_{2i}+\alpha_3 x_{3i}+ \delta_i + \varepsilon_i$
- Examples
  - Age, IQ, brain volume, cortex thickness
  - Behavioral data

Handling covariates: one group

- Centering: tricky business
  - $y_i = \alpha_0 + \alpha_1 x_i + \delta_i + \varepsilon$, for ith subject
  - Interested in group effect α_0 (x=0) while controlling (partialling out) x
  - α_1 - slope (change rate): % signal change per unit of x
  - Interpretability: group effect α_0 at what value of x: mean or any other value?

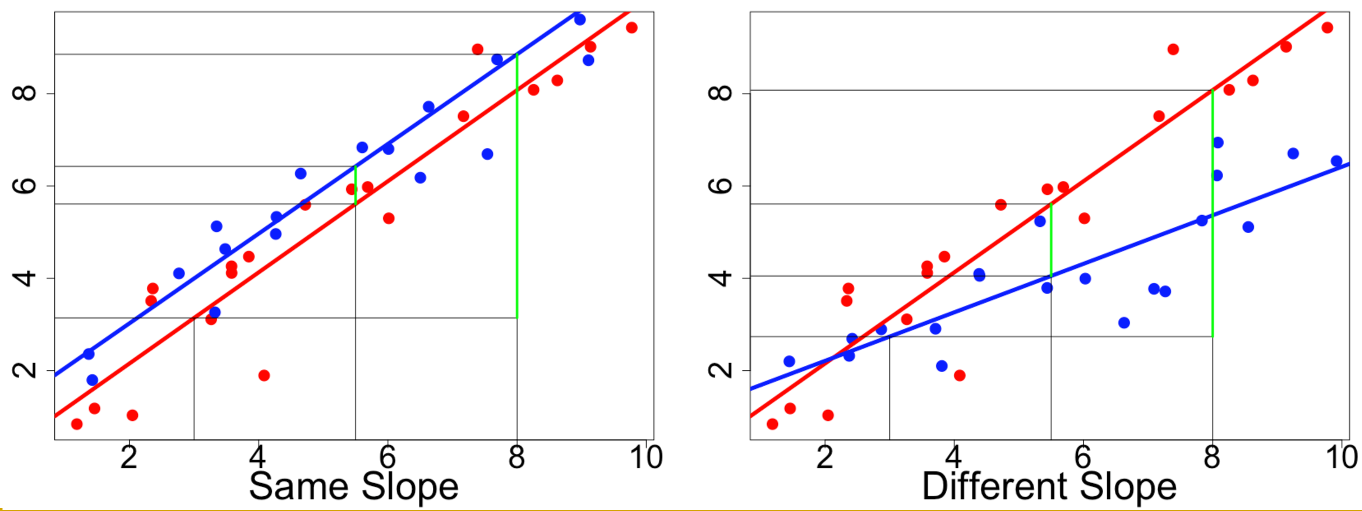


Covariates: trickier with > 1 group

- Center and slope
  - $y_i = \alpha_0 + \alpha_1 x_{1i} + \alpha_2 x_{2i} + \alpha_3 x_{3i} + \delta_i + \varepsilon$, for *i*th subject
    - x_1: group indicator
    - x_2: covariate
    - x_3: group effect in slope (interaction btw group and covariate)
  - What we're interested
    - Group effects α_0 and α_1 while controlling covariate
  - Interpretability
    - Center
      - Group effect α_0 and α_1 at what covariate value?
      - Same or different center across groups?
    - Slope
      - same ($\alpha_3=0$) or different ($\alpha_3 \neq 0$) slope across groups

Covariates: scenarios with 2 groups

- Center and slope
  - $y_i = \alpha_0 + \alpha_1 x_{1i} + \alpha_2 x_{2i} + \alpha_3 x_{3i} + \delta_i + \varepsilon_i$, for ith subject
  - Interpretability
    - Same center and slope ($\alpha_3=0$)
    - Different center with same slope ($\alpha_3=0$)
    - Same center with different slope ($\alpha_3 \neq 0$)
    - Different center and slope ($\alpha_3 \neq 0$)



Start simple: one-sample test

- Random-effects: $y_i = \theta_i + \varepsilon_i = \alpha_0 + \delta_i + \varepsilon_i$, for ith subject
  - y_i : β or linear combination (contrast) of β's from ith subject
  - $\theta_i = \alpha_0 + \delta_i$: "true" individual effect from ith subject
  - α_0: group effect we'd like to find out
  - δ_i : deviation of ith subject from group effect α_0, $N(0, \tau^2)$
  - ε_i: sample error from ith subject, $N(0, \sigma_i^2)$, σ_i^2 known!
- Special cases
  - $\sigma_i^2=0$ reduced to conventional group analysis: One-sample t: $y_i = \alpha_0 + \delta_i$
  - $\delta_i=0$ ($\tau^2=0$) assumed in fixed-effects (FE) model: Ideally we could find out all possible explanatory variables so only an FE model is necessary!
- Mature meta analysis tools for this simple model
  - Broadly used in clinical trials/epidemiology in recent 20 yrs
  - A special case of linear mixed-effects model

MEMA with one-sample test

- Random-effects: $y_i = \alpha_0 + \delta_i + \varepsilon_i$, for ith subject
  - $\delta_i \sim N(0, \tau^2)$, $\varepsilon_i \sim N(0, \sigma_i^2)$, σ_i^2 known, τ^2 unknown
  - What can we achieve?
    - Null hypothesis about group effect H_0: $\alpha_0 = 0$
    - Checking group heterogeneity H_0: $\tau^2 = 0$
    - Any outliers among the subjects? Adding some confounding variable(s)? Grouping subjects?
  - We know σ_i^2, and pretend we also knew τ^2, weighted least squares (WLS) gives
    - The "best" estimate $\hat{\alpha}_0 = \dfrac{\sum w_i y_i}{\sum w_i}, w_i = \dfrac{1}{\tau^2 + \sigma_i^2}$
    - **BLUE**: unbiased with minimum variance
  - Wake up: Unfortunately we don't know τ^2!!!

Solving MEMA in one-sample case

- Estimating τ^2: a few approaches
  - Method of moment (MoM) - DSL
  - Maximum likelihood (ML)
  - Restricted/residual/reduced/marginal ML (REML): 3dMEMA
- Statistical testing
  - Group effect α_0=0: $Z = \frac{\sum w_i y_i}{\sqrt{\sum w_i}} \cong N(0,1), w_i = \frac{1}{\tau^2 + \sigma_i^2}$
    - Wald or Z-test: assume enough subjects with normal distributions
    - Go with t-test when in doubt
  - Heterogeneity test τ^2=0: $Q = \sum_{i=1}^{n} \frac{(y_i - \hat{\alpha}_0)^2}{\sigma_i^2} \sim \chi^2(n-1)$
  - Outlier identification for each subject through Z-statistic

We don't limit ourselves to simple case

- $y_i = \alpha_0 + \alpha_1 x_{i1} + \ldots + \alpha_{ip} x_{ip} + \delta_i + \varepsilon_i$, for ith subject
  - Mixed-effects model or meta regression
  - y_i: β or linear combination (contrast) of β's from ith subject
  - α_0: common group effect we'd like to find out
  - x_{ij}: an indicator/dummy variable showing, for example, group to which ith subject belongs, level at which a factor lies, or a continuous variable such as covariate (e.g., age, IQ) (j=1,…,p)
  - δ_i : deviation of ith subject from group effect α_0, $N(0, \tau^2)$
  - ε_i: sample error from ith subject, $N(0, \sigma_i^2)$, σ_i^2 known!
- Combine subjects into a concise model in matrix form
  - $\mathbf{y}_{n\times 1} = \mathbf{X}_{n\times p}\alpha_{p\times 1} + \boldsymbol{\delta}_{n\times 1} + \boldsymbol{\varepsilon}_{n\times 1}$
  - $\mathbf{y} \sim N(\mathbf{X}\alpha, \tau^2\mathbf{I}_n + \mathbf{V})$, $\mathbf{V} = \text{diag}(\sigma_1, \ldots, \sigma_n)$ known, τ^2 unknown
  - Estimate α and τ^2 simultaneously via maximizing REML

Dealing with outliers

- Detection
  - Ideally we wish to account for anything until having no cross-subject variability: $\tau^2 = 0$!
  - 4 quantities to check cross-subject variability
    - Cross subject variability (heterogeneity) τ^2
    - Q for H_0: $\tau^2 = 0$
    - Intra-class correlation (ICC): $\lambda = \sigma_i^2/(\sigma_i^2+\tau^2)$
    - Z statistic of ε_i
- Modeling: how to handle outliers in the model?
  - Ignore those subjects with 2 s.d. away from mean?
    - Arbitrary: OK with data within 1.9 s.d.?
    - How about when outliers occur at voxel level?
    - If throwing away outliers at voxel level, varying DFs across brain?

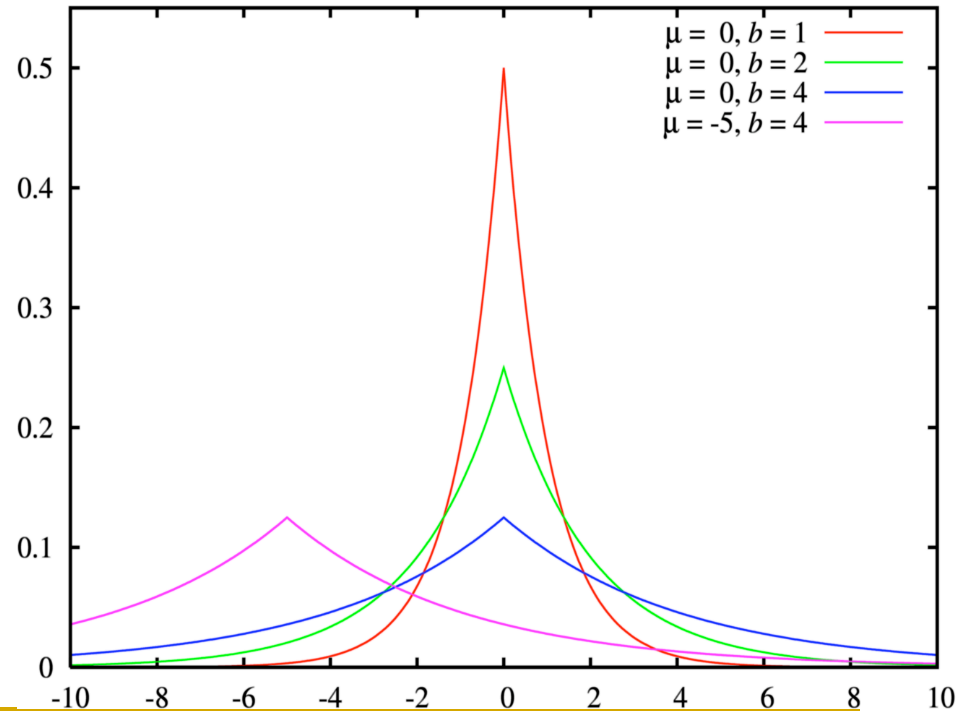
Modeling outliers

- Modeling: how to handle outliers in the model?
  - Typically a Gaussian for subject deviation: $\delta_i \sim N(0, \tau^2)$
  - With outliers, assume a Laplace (double exponential) distribution

$$f(x|\mu,b) = \frac{1}{2b} \exp\left(-\frac{|x-\mu|}{b}\right)$$

- μ: location parameter
- b: scale parameter
- Mean=median=mode=μ
- Variance = $2b^2$
- Fatter tail but smaller Var
- Estimator of μ is sample median, and ML estimator of b

$$\hat{b} = \frac{1}{N}\sum_{i=1}^{N}|x_i - \hat{\mu}|$$



Modeling outliers

- Laplace distribution for outlier modeling
  - No REML form
  - Go with ML: variance estimate τ^2 might be slightly underestimated
  - Computation cost: higher
  - Generally higher statistical power

Moral of a story

- Story
  - Strong activation at individual level and in ROI analysis failed to show up at group level
  - Result with 3dMEMA showed consistency with individual and ROI analysis
  - Magic power of 3dMEMA? Relatively robust to some (unreliable) outliers
- Check brick labels for all input files

```
foreach subj (S1 S2 S3 …)
   3dinfo -verb ${subj}_file+tlrc  | grep 'sub-brick #0'
end

++ 3dinfo: AFNI version=AFNI_2008_07_18_1710 (Jul  8 2009) [32-bit]
 -- At sub-brick #0 'contr_GLT#0_Coef' datum type is float:     -0.78438 to      0.867817
 -- At sub-brick #0 'contr_GLT#0_Coef' datum type is float:    -0.444093 to      0.501589
…
```

Suggested preprocessing steps

- Input
  - β and *t*-statistic from each subject
  - One sub-brick per input file (3dbucket)
- Some suggestions
  - Slice timing correction and volume registration
  - Aligning/warping to standard space
    - Avoid troubling step of warping on *t*-statistic
  - Smoothing: 3dBlurToFWHM
  - Scaling
  - All input files, β and more importantly *t*-statistic, come from 3dREMLfit instead of 3dDeconvolve
  - No masking applied at individual level so that no data is lost at group level along the edge of (and sometimes inside) the brain

Comparisons among FMRI packages

Program	Language	Algorithm	Runtime	Group effect statistics	Covariates	Voxelwise outlier detection	Voxelwise outlier modeling
multistat (FMRIstat)	Matlab	EM for REML + spatial regularization	~1 min per test	t	✗	✗	✗
FLAME in FEAT (FSL)	C/C++	Bayesian + MCMC	45-200 min per test + threshold	fitted with t	✔	% subjects for group, p for each subject	mixture of two Gaussian
3dMEMA (AFNI)	R	ML/REML/ MoM	3-15 min per test	Z/t	✔	τ^2 + Q for group, λ + Z for each subject	Laplace

Overview: 3dMEMA

- http://afni.nimh.nih.gov/sscc/gangc/MEMA.html
- Meta analysis: compromise between Bayesian and frequentist
  - Backbone: WLS + maximization of REML or ML of Laplace-Gauss
  - Currently available types
    - One-, two-, paired-sample test
    - Covariates allowed: careful with centering and interaction with groups
  - Output
    - Group level: group effect (% sigmal change) and statistics (*Z*/*t*), cross-subject heterogeneity τ^2 and Q (χ^2-test)
    - Individual level: λ + *Z* for each subject
  - Generally more powerful/valid than conventional approach
  - Relatively robust against most outliers
  - Moderate computation cost with parallel computing: 3-20 minutes
- Limitations
  - Can't handle sophisticated types: multiple basis functions; *F*-test types
  - Computation cost