Group Analysis

File: **afni24_GroupAna.pdf**

Gang Chen

SSCC/NIMH/NIH/HHS

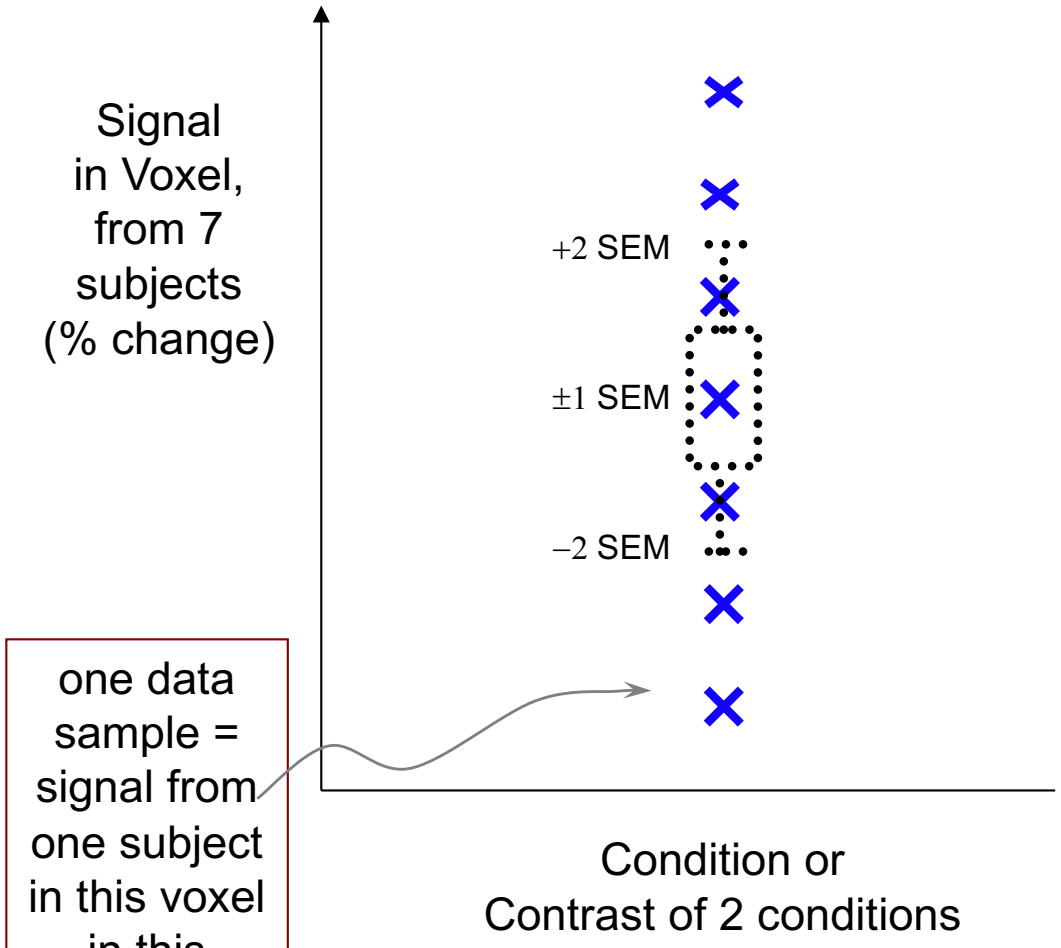
Preview

- Introduction: basic concepts and terminology
  - Why do we need to do group analysis?
  - Factor, quantitative covariates, main effect, interaction, …
- Group analysis approaches
  - *t*-test: 3dttest++ (3dttest), 3dMEMA
  - Regression: 3dttest++, 3dMEMA, 3RegAna
  - ANOVA: 3dANOVAx, 3dMVM, GroupAna
  - ANCOVA or GLM: 3dttest++, 3dMEMA, 3dMVM, 3dLME
  - Impact & consequence of FSM, ASM, and ESM
- Miscellaneous
  - Centering for covariates
  - Intra-Class Correlation (ICC)
  - Nonparametric approach, fixed-effects analysis
  - Inter-Subject Correlation (ISC)

Why Group Analysis?

- Evolution of FMRI studies
  - Early days [1992-1994]: no need for group analysis
    - Seed-based correlation for one subject was revolutionary
  - Now: torture brain/data enough, and hope nature will confess!
    - Many ways to manipulate the brain (and data)
- Reproducibility and generalization
  - **Science strives for generality**: summarizing subject results
  - Typically 10 or more subjects per group
  - Exceptions: pre-surgical planning, lie detection, …
- Why not one analysis with a giant model for all subjects?
  - Computationally unmanageable and very hard to set up
  - Heterogeneity in data or experiment design across subjects
  - Model and data quality check at individual subject level

Simplest Group Analysis: One-Sample *t*-Test

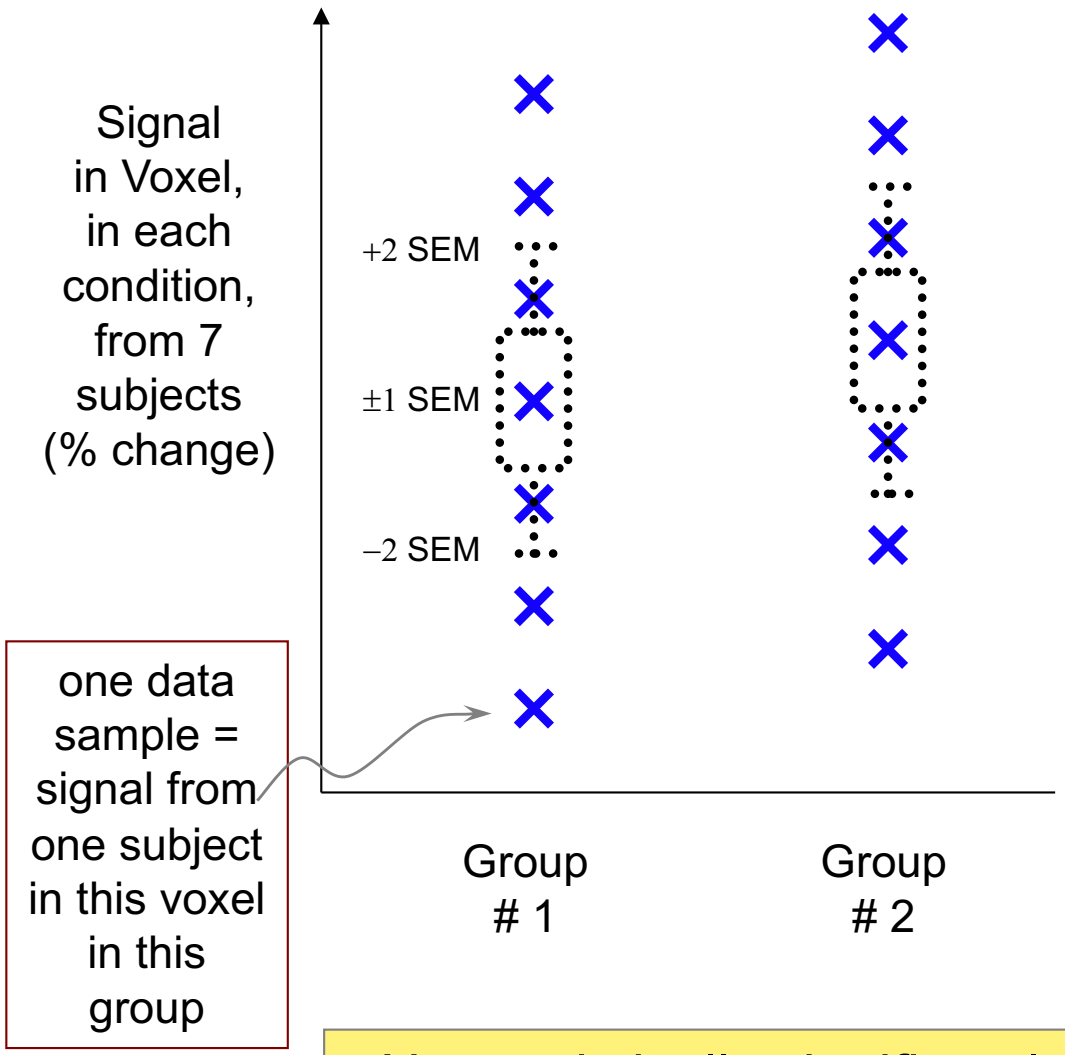


- SEM = Standard Error of the Mean
= standard deviation of sample, divided by square root of number of samples
= estimate of uncertainty in sample mean

- One-sample *t*-test determines if sample mean is large enough relative to SEM

- statistically significantly different from 0!

Simplest Group Analysis: Two-Sample *t*-Test



- Group = some way to categorize subjects (*e.g.*, sex, drug treatment, disease, …)

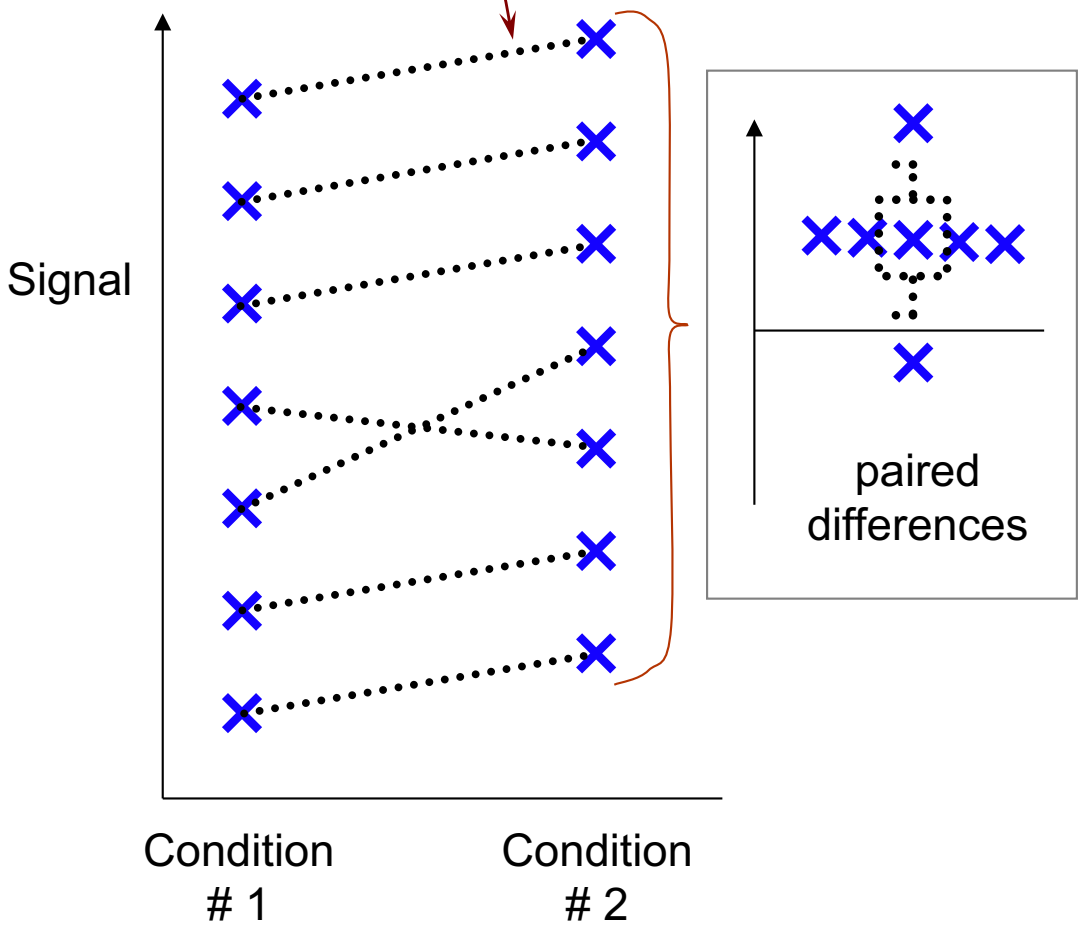
- SEM = Standard Error of the Mean = standard deviation of sample divided by square root of number of samples
= estimate of uncertainty in sample mean

- Two-sample *t*-test determines if sample means are "far apart" compared to size of SEM

- Not statistically significantly different!

Simplest Group Analysis: Paired (~1-sample) *t*-Test

paired data samples: same numbers as before



- Significantly different!
- Condition #2 > #1, per subject

- Paired means that samples in different conditions should be linked together (*e.g.*, from same subjects)
- Test determines if differences between conditions in each pair are "large" compared to SEM of the differences
- Paired test can detect systematic *intra*-subject differences that can be hidden in *inter*-subject variations
- Lesson: properly separating ***inter***-subject and ***intra***-subject signal variations can be very important!
- **Essentially equivalent to one-sample *t*-test**

Toy example of group analysis

- Responses from a group of subjects under one condition
  - : $(\beta_1, \beta_2, \ldots, \beta_{10})=(1.13, 0.87, \ldots, 0.72)$ [% signal change]
- Centroid: average $(\beta_1+\beta_2+\ldots+\beta_{10})/10 = 0.92$ is not enough
  - Variation/reliability measure: diversity, spread, deviation
  - How different is 0.92 from 0 compared to its deviation?
- Model building
  - Subject *i*'s response = group average + deviation of subject *i*: simple model GLM (one-sample *t*-test)

$$\hat{\beta}_i = b + \epsilon_i, \epsilon_i \sim N(0, \sigma^2)$$

  - If individual responses are consistent, ϵ_i should be small
  - How small (*p*-value)?
    - *t*-test: significance measure = $\hat{b} / (\hat{\sigma} / n)$
- 2 measures: ***b*** (dimensional) and ***t*** (dimensionless)

Group Analysis Caveats

- Results: two components (in afni GUI: OLay + Thr)
  - Effect estimates: have unit and physical meaning
  - Their significance (response to house **significantly** > face)
    - Very unfortunately *p*-values solely focused in FMRI!
- Statistical significance (*p*-value) becomes obsession
  - Published papers: Big and tall parents (violent men, engineers) have more sons, beautiful parents (nurses) have more daughters
  - Statistical significance is not the same as practical importance
- **Fallacy**: **binarized thinking** -- an effect that fails to reach statistical significance is not necessarily nonexistent
  - Statistically insignificant effect might be real
  - Sample size, suboptimal model, poor alignment across subjects

Group Analysis Caveats

- Conventional: voxel-wise (brain) or node-wise (surface)
  - Prerequisite: reasonable alignment to some template
  - Limitations: alignment could be suboptimal or even poor
    - Different folding patterns across subjects: better alignment could help (perhaps to 5 mm accuracy?)
    - Different cytoarchitectonic (or functional) locations across subjects: structural alignment of images won't help!
    - Impact on conjunction vs. selectivity
- Alternative (won't discuss): ROI-based approach
  - Half data for functional localizers, and half for ROI analysis
  - Easier: whole brain reduced to a few numbers per subject
  - Model building and tuning possible
  - Most AFNI 3d analysis programs also handle ROI input (1D files)

Group Analysis in NeuroImaging: why big models?

- Various group analysis approaches
  - Student's ***t*-test**: one-, two-sample, and paired
  - **ANOVA**: one or more categorical explanatory variables (factors)
  - **GLM**: AN(C)OVA
  - **LME**: linear mixed-effects modeling
- Easy to understand: *t*-tests not always practical or feasible
  - Tedious when layout (structure of data) is too complex
  - Main effects and interactions: desirable
  - Controlling for quantitative covariates
- Advantages of big models: AN(C)OVA, GLM, LME
  - All tests in one analysis (vs. piecemeal *t*-tests): omnibus *F*
  - Controlling for covariate effects
  - Power gain: combining subjects across groups for estimates of signal *and* noise parameters (*i.e.,* variances and correlations)

Terminology: Explanatory variables

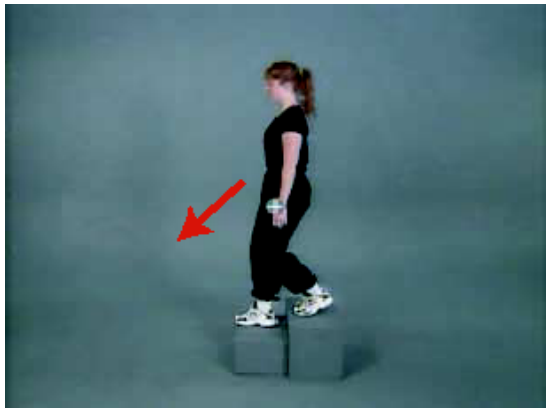
- **Response/Outcome variable** (HDR): regression β coefficients
- **Factor**: categorical, qualitative, descriptive, nominal, or discrete
  - Categorization of conditions/tasks
    - Within-subject (repeated-measures) factor
  - Subject-grouping: group of subjects
    - Between-subjects factor
    - Gender, patients/controls, genotypes, handedness, …
  - Subject: random factor measuring deviations
    - Of no interest, but served as random samples from a population
- **Quantitative** (numeric or continuous) **covariate**
  - Three usages of 'covariate'
    - Quantitative value (rather than strict separation into groups)
    - Variable of no interest: qualitative (scanner, sex, handedness) or quantitative
    - Explanatory variable (regressor, independent variable, or predictor)
  - Examples: age, IQ, reaction time, brain volume, …

Terminology: Fixed effects

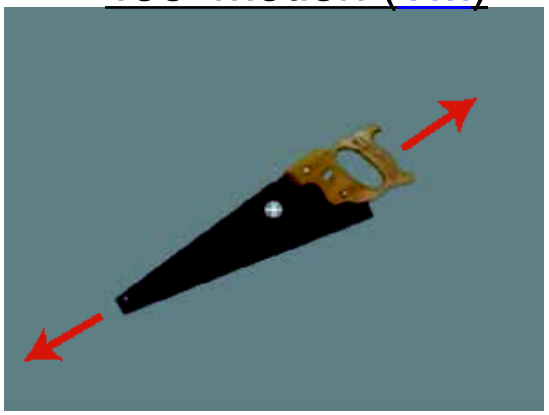
- Fixed-effects factor: categorical (qualitative or discrete) variable
  - Treated as a fixed variable (constant to be estimated) in the model
    - Categorization of conditions/tasks (modality: visual/auditory)
      - Within-subject (repeated-measures) factor: 3 emotions
    - Subject-grouping: Group of subjects (gender, controls/patients)
      - Between-subject factor
  - All factor levels are of interest: **not interchangeable/replaceable**
    - main effect, contrasts among levels
  - Fixed in the sense of statistical inferences
    - Apply only to the specific levels of the factor: : **replacement test**
      - Categories: human, tool
    - Don't extend to other potential levels that might have been included (but were not)
      - Inferences from viewing human and tool categories can't be generated to animals or clouds or Martians
- Fixed-effects variable: quantitative covariate

Remember This Study?

Human whole-body motion (HM)



Tool motion (TM)



Human point motion (HP)

From Figure 1
Beauchamp et al. 2003

Tool point motion (TP)

2 Factors, each with 2 levels

- **Factor A = type of object being viewed**
  - **Levels = Human or Tool**
- **Factor B = type of display seen by subject**
  - **Levels = Whole or Points**
- **This is repeated measures (4 βs per subject), 2×2 factorial**

Terminology: Random effects

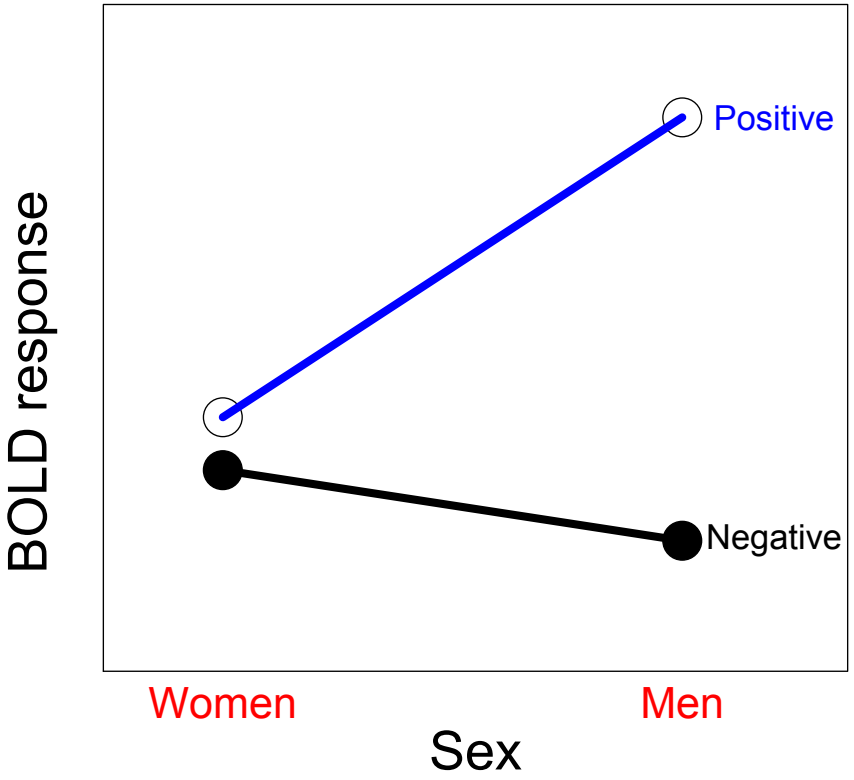
- Random factor/effect
  - Random variable in the model: exclusively used for subject in FMRI
    - average + effects attributable to each subject: *e.g.* $N(\mu, \tau^2)$
    - Requires enough subjects to estimate properly
  - Each individual subject effect is of NO interest: **replacement test**
    - Group response = 0.92%, subject 7 = 1.13%, random effect = 0.21%
  - Random in the sense
    - Subjects as random samples (representations) from a population
    - Inferences can be generalized to a hypothetical population
- A generic group model: decomposing each subject's response
  - Fixed (population) effects: universal constants (immutable): $\boldsymbol{\beta}$

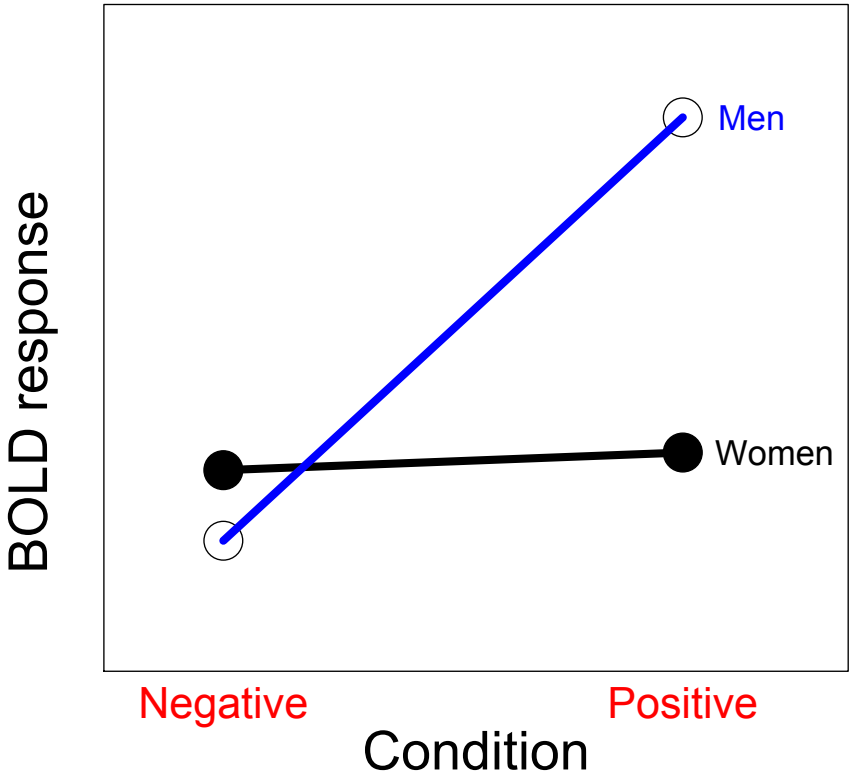
$$\boldsymbol{y}_i = X_i\boldsymbol{\beta} + Z_i\boldsymbol{b}_i + \boldsymbol{\epsilon}_i$$

  - Random effects: individual subject's deviation from the population (personality: durable for subject i): $\boldsymbol{b}_i$
  - Residuals: noise (evanescent): $\boldsymbol{\varepsilon}_i$

Terminology: Omnibus tests - main effect and interaction

- Main effect: any difference across levels of a factor?
- Interactions: with ≥ 2 factors, interaction may exist
  - 2 × 2 design: *F*-test for interaction between A and B = *t*-test of (A1B1 - A1B2) - (A2B1 - A2B2) or (A1B1 - A2B1) - (A1B2 - A2B2)
    - *t* stastistic is better than *F* : a positive *t* shows A1B1 - A1B2 > A2B1 - A2B2 and A1B1 - A2B1 > A1B2 - A2B2



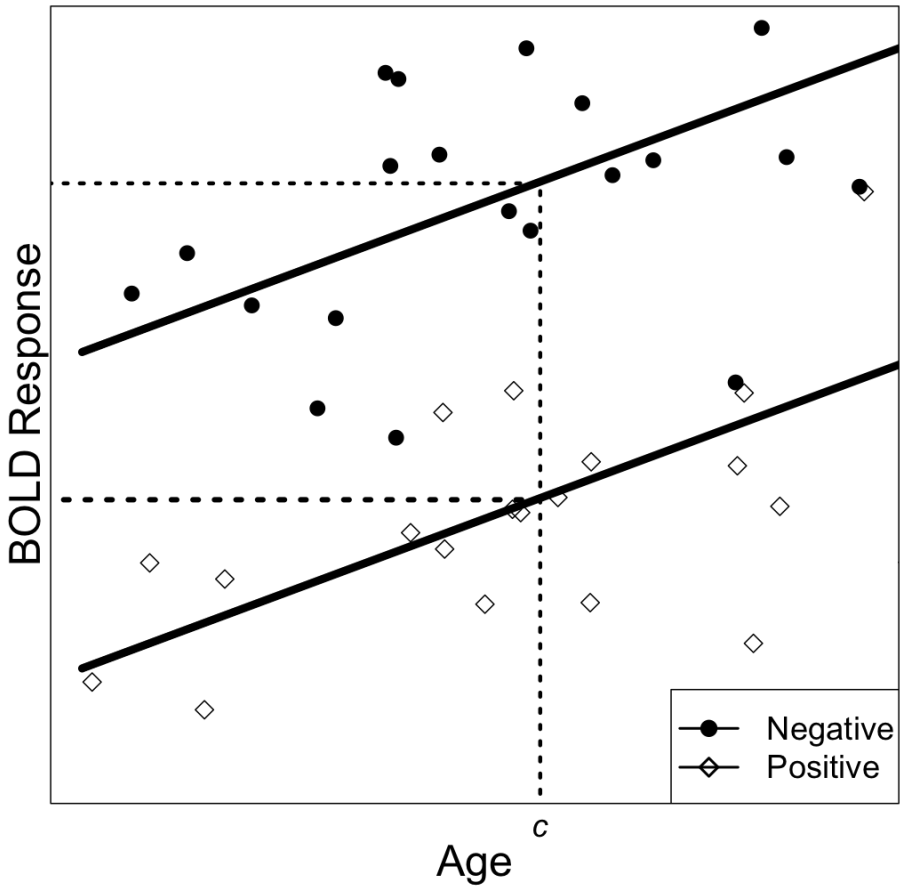


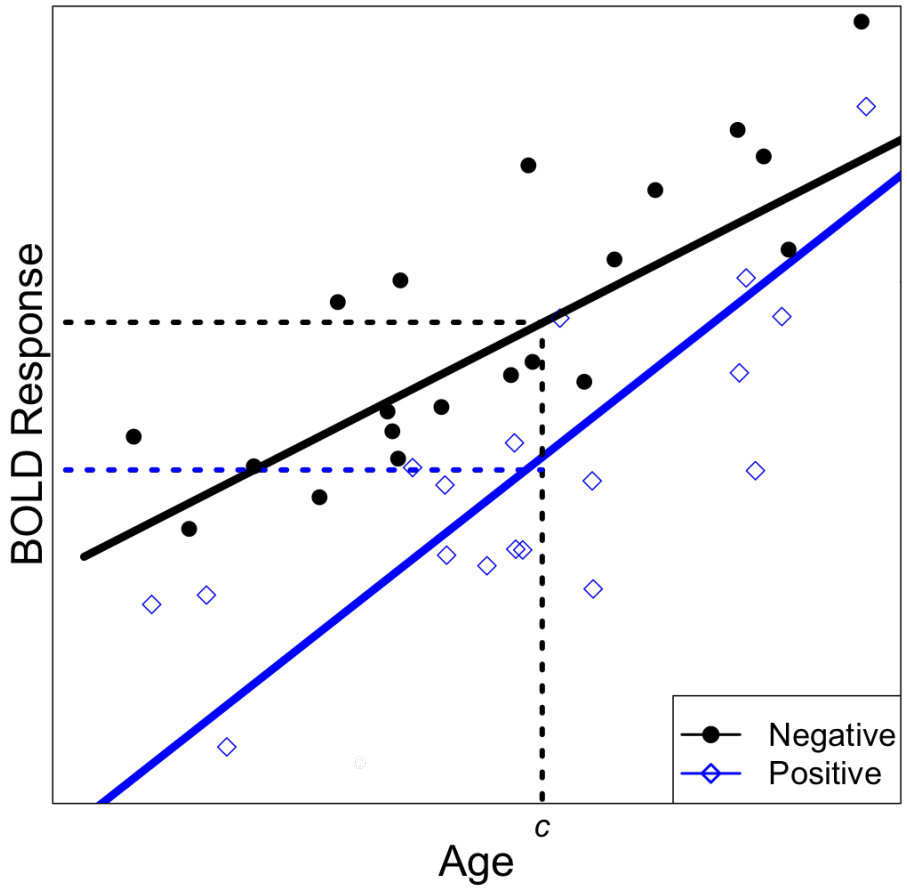
Terminology: Interaction

- Interactions: ≥ 2 factors
  - May become very tedious to sort out or understand!
    - ≥ 3 levels in a factor
    - ≥ 3 factors
  - Solutions: reduction (in complexity)
    - Pairwise comparison
    - Plotting: ROI averages
  - Requires sophisticated modeling
    - AN(C)OVA: 3dANOVAx, 3dMVM, 3dLME
- Interactions: quantitative covariates
  - In addition to linear effects, may have nonlinearity: y might depend on products of covariates: $x_1 * x_2$, or x^2

Terminology: Interaction

- Interaction: between a factor and a quantitative covariate





  - Using explanatory variable (Age) in a model as a nuisance regressor (additive effect) may not be enough
    - Model building/tuning: Potential interactions with other explanatory variables? (as in graph on the right)
    - Of scientific interest (*e.g.*, gender differences)

Models at Group Level

- Conventional approach: taking β (or linear combination of multiple βs) only for group analysis
  - Assumption: all subjects have same precision (reliability, standard error, confidence interval) about β
  - All subjects are treated equally
  - Student *t*-test: paired, 1- and 2-sample: *not* random-effects models in strict sense (said to be random effects in *S*ome other *P*rogra*M*)
  - AN(C)OVA, GLM, LME
- More precise method: taking both effect estimates and *t*-stats
  - *t*-statistic contains precision information about effect estimates
  - Each subject's β is weighted based on precision of effect estimate (more precise βs get more weight)
  - Currently only available for *t*-test types

Piecemeal *t*-tests: 2 × 3 Mixed ANCOVA example

- A relatively simple model, but **challenging** for neuroimaging
  - Factor A (Group): 2 levels (patient and control)
  - Factor B (Condition): 3 levels (pos, neg, neu)
  - Factor S (Subject): 15 ASD children and 15 healthy controls
  - Quantitative covariate: Age
- Using Multiple *t*-tests for this study
  - Group comparison + age effect
  - Pairwise comparisons among three conditions
    - Cannot control for age effect
  - Effects that cannot be analyzed as *t*-tests
    - Main effect of Condition (3 levels is beyond *t*-test method)
    - Interaction between Group and Condition (6 levels total)
    - Age effect across three conditions (just too complicated)

Classical ANOVA: 2 × 3 Mixed ANOVA

- Factor A (Group): 2 levels (patient and control)
- Factor B (Condition): 3 levels (pos, neg, neu)
- Factor S (Subject): 15 ASD children and 15 healthy controls
- Covariate (Age): **cannot** be modeled; **no** correction for sphericity violation

$$F_{(a-1,a(n-1))}(A) = \frac{MSA}{MSS(A)},$$

$$F_{(b-1,a(b-1)(n-1))}(B) = \frac{MSB}{MSE},$$

$$F_{((a-1)(b-1),a(b-1)(n-1))}(AB) = \frac{MSAB}{MSE}$$

Different denominator

where

$$MSA = \frac{SSA}{a-1} = \frac{1}{a-1}\left(\frac{1}{bn}\sum_{j=1}^{a} Y_{.j.}^2 - \frac{1}{abn}Y_{...}^2\right),$$

$$MSB = \frac{SSB}{b-1} = \frac{1}{b-1}\left(\frac{1}{an}\sum_{k=1}^{b} Y_{..k}^2 - \frac{1}{abn}Y_{...}^2\right),$$

$$MSAB = \frac{SSAB}{(a-1)(b-1)} = \frac{1}{(a-1)(b-1)}\left(\frac{1}{n}\sum_{j=1}^{a}\sum_{k=1}^{b} Y_{.jk} - \frac{1}{bn}\sum_{j=1}^{a} Y_{.j.}^2 - \frac{1}{an}\sum_{k=1}^{b} Y_{..k}^2 + \frac{1}{abn}Y_{...}^2\right),$$

$$MSS(A) = \frac{SSS(A)}{a(n-1)} = \frac{1}{a(n-1)}\left(\frac{1}{b}\sum_{i=1}^{n}\sum_{j=1}^{a} Y_{ij.}^2 - \frac{1}{bn}\sum_{j=1}^{a} Y_{.j.}^2\right),$$

$$MSE = \frac{1}{a(b-1)(n-1)}\left(\sum_{i=1}^{n}\sum_{j=1}^{a}\sum_{k=1}^{b} Y_{ijk}^2 - \frac{1}{n}\sum_{j=1}^{a}\sum_{k=1}^{b} Y_{.jk} - \frac{1}{b}\sum_{i=1}^{n}\sum_{j=1}^{a} Y_{ij.}^2 + \frac{1}{bn}\sum_{j=1}^{a} Y_{.j.}^2 + \frac{1}{abn}Y_{...}^2\right)$$

Univariate GLM: 2 x 3 mixed ANOVA

- Group: 2 levels (patient and control)
- Condition: 3 levels (pos, neg, neu)
- Subject: 3 ASD children and 3 healthy controls

Difficult to incorporate covariates
- Broken orthogonality of matrix

No correction for sphericity violation

$$
\begin{array}{c}\text{Subj}\\ 1\\1\\1\\2\\2\\2\\3\\3\\3\\4\\4\\4\\5\\5\\5\\6\\6\\6\end{array}
\begin{pmatrix}\beta_{11}\\\beta_{12}\\\beta_{13}\\\beta_{21}\\\beta_{22}\\\beta_{23}\\\beta_{31}\\\beta_{32}\\\beta_{33}\\\beta_{41}\\\beta_{42}\\\beta_{43}\\\beta_{51}\\\beta_{52}\\\beta_{53}\\\beta_{61}\\\beta_{62}\\\beta_{63}\end{pmatrix}
=
\begin{array}{c}
\begin{array}{cccccccccc} X_0 & X_1 & X_2 & X_3 & X_4 & X_5 & X_6 & X_7 & X_8 & X_9 \end{array}\\
\begin{pmatrix}
1 & 1 & 1 & 0 & 1 & 0 & 1 & 0 & 0 & 0\\
1 & 1 & 0 & 1 & 0 & 1 & 1 & 0 & 0 & 0\\
1 & 1 & -1 & -1 & -1 & -1 & 1 & 0 & 0 & 0\\
1 & 1 & 1 & 0 & 1 & 0 & 0 & 1 & 0 & 0\\
1 & 1 & 0 & 1 & 0 & 1 & 0 & 1 & 0 & 0\\
1 & 1 & -1 & -1 & -1 & -1 & 0 & 1 & 0 & 0\\
1 & 1 & 1 & 0 & 1 & 0 & -1 & -1 & 0 & 0\\
1 & 1 & 0 & 1 & 0 & 1 & -1 & -1 & 0 & 0\\
1 & 1 & -1 & -1 & -1 & -1 & -1 & -1 & 0 & 0\\
1 & -1 & 1 & 0 & -1 & 0 & 0 & 0 & 1 & 0\\
1 & -1 & 0 & 1 & 0 & -1 & 0 & 0 & 1 & 0\\
1 & -1 & -1 & -1 & 1 & 1 & 0 & 0 & 1 & 0\\
1 & -1 & 1 & 0 & -1 & 0 & 0 & 0 & 0 & 1\\
1 & -1 & 0 & 1 & 0 & -1 & 0 & 0 & 0 & 1\\
1 & -1 & -1 & -1 & 1 & 1 & 0 & 0 & 0 & 1\\
1 & -1 & 1 & 0 & -1 & 0 & 0 & 0 & -1 & -1\\
1 & -1 & 0 & 1 & 0 & -1 & 0 & 0 & -1 & -1\\
1 & -1 & -1 & -1 & 1 & 1 & 0 & 0 & -1 & -1
\end{pmatrix}
\end{array}
\begin{pmatrix}\alpha_0\\\alpha_1\\\alpha_2\\\alpha_3\\\alpha_4\\\alpha_5\\\alpha_6\\\alpha_7\\\alpha_8\\\alpha_9\end{pmatrix}
+
\begin{pmatrix}\delta_{11}\\\delta_{12}\\\delta_{13}\\\delta_{21}\\\delta_{22}\\\delta_{23}\\\delta_{31}\\\delta_{32}\\\delta_{33}\\\delta_{41}\\\delta_{42}\\\delta_{43}\\\delta_{51}\\\delta_{52}\\\delta_{53}\\\delta_{61}\\\delta_{62}\\\delta_{63}\end{pmatrix}
$$

b = X a + d

Univariate GLM: popular in neuroimaging

- Advantages: more *flexible* than the method of sums of squares
  - No limit on the the number of explanatory variables (in principle)
  - Easy to handle unbalanced designs
  - Covariates easily modeled *when* no within-subject factors present
- Disadvantages: costs paid for the flexibility
  - Intricate dummy coding (to allow for different factors and levels)
  - Tedious pairing for numerator and denominator of *F*-stat
    - Choosing proper denominator SS is not obvious (errors in some software)
    - Can't generalize (in practice) to any number of explanatory variables
    - Susceptible to invalid formulations and problematic post hoc tests
  - **Cannot** handle covariates in the presence of within-subject factors
  - **No** direct approach to correcting for sphericity violation
    - **Unrealistic** assumption: **same** variance-covariance structure
- **Problems**: When overall residual SS is adopted for all tests
  - *F*-stat: valid only for **highest** order interaction of within-subject factors
  - Most post hoc tests are inappropriate with this denominator

Univariate GLM: **problematic implementations**

- Between-subjects Factor A (Group): 2 levels (patient, control)
  Within-subject Factor B (Condition): 3 levels (pos, neg, neu)

  A) Omnibus tests

Correct

$$F_A = \frac{MSA}{MSA(C)},$$

$$F_B = \frac{MSB}{MSE},$$

$$F_{AB} = \frac{MSAB}{MSE}$$

Incorrect

$$F_A = \frac{MSA}{MSE},$$

$$F_B = \frac{MSB}{MSE},$$

$$F_{AB} = \frac{MSAB}{MSE}$$

  B) Post hoc tests (contrasts)

  (1) **Incorrect** *t*-tests for factor A due to incorrect denominator

  (2) **Incorrect** *t*-tests for factor B or interaction effect AB when weights do not add up to 0

  C) How to handle multiple *β*s per effect (e.g., multiple runs)?

  -- **Artificially inflated DOF and assumption violation** when multiple *β*s are fed into program

Univariate GLM: **problematic implementations**

- Within-subjects Factor A (Object): 2 levels (house, face)
  Within-subject Factor B (Condition): 3 levels (pos, neg, neu)

  A) Omnibus tests

Correct

$$F_A = \frac{MSA}{MSAC},$$

$$F_B = \frac{MSB}{MSBC},$$

$$F_{AB} = \frac{MSAB}{MSE}$$

Incorrect

$$F_A = \frac{MSA}{MSE},$$

$$F_B = \frac{MSB}{MSE},$$

$$F_{AB} = \frac{MSAB}{MSE}$$

  B) Post hoc tests (contrasts)

  (1) **Incorrect** *t*-tests for both factors A and B due to incorrect denominator

  (2) **Incorrect** *t*-tests for interaction effect AB if weights don't add up to 0

  C) How to handle multiple *β*s per effect (e.g., multiple runs)?

  -- **Artificially inflated DOF and assumption violation** when multiple *β*s are fed into program

Better Approach: Multivariate GLM

- Group: 2 levels (patient and control)
- Condition: 3 levels (pos, neg, neu)
- Subject: 3 ASD children and 3 healthy controls
- Age: quantitative covariate

$$\boldsymbol{B}_{n\times m} = \boldsymbol{X}_{n\times q}\,\boldsymbol{A}_{q\times m} + \boldsymbol{D}_{n\times m}$$

$$\begin{array}{c} Subj \\ 1 \\ 2 \\ 3 \\ 4 \\ 5 \\ 6 \end{array}\begin{array}{c} \begin{pmatrix} Pos & Neg & Neu \\ \beta_{11} & \beta_{12} & \beta_{13} \\ \beta_{21} & \beta_{22} & \beta_{23} \\ \beta_{31} & \beta_{32} & \beta_{33} \\ \beta_{41} & \beta_{42} & \beta_{43} \\ \beta_{51} & \beta_{52} & \beta_{53} \\ \beta_{61} & \beta_{62} & \beta_{63} \end{pmatrix} \\ B \end{array} = \begin{array}{c} \begin{pmatrix} Int & Grp & Age \\ 1 & 1 & -6 \\ 1 & 1 & 10 \\ 1 & 1 & 4 \\ 1 & -1 & -4 \\ 1 & -1 & -1 \\ 1 & -1 & -3 \end{pmatrix} \\ X \end{array} \begin{array}{c} \begin{pmatrix} Pos & Neg & Neu \\ \alpha_{01} & \alpha_{02} & \alpha_{03} \\ \alpha_{11} & \alpha_{12} & \alpha_{13} \\ \alpha_{21} & \alpha_{22} & \alpha_{23} \end{pmatrix} \\ A \end{array} + \begin{array}{c} \begin{pmatrix} Pos & Neg & Neu \\ \delta_{11} & \delta_{12} & \delta_{13} \\ \delta_{21} & \delta_{22} & \delta_{23} \\ \delta_{31} & \delta_{32} & \delta_{33} \\ \delta_{41} & \delta_{42} & \delta_{43} \\ \delta_{51} & \delta_{52} & \delta_{53} \\ \delta_{61} & \delta_{62} & \delta_{63} \end{pmatrix} \\ D \end{array} \begin{array}{c} Subj \\ 1 \\ 2 \\ 3 \\ 4 \\ 5 \\ 6 \end{array}$$

Why use β , not *t*, values for group analysis?

- Why not use individual level statistics (*t*, *F*)?
  - Dimensionless, no physical meaning
  - Sensitive to sample size (number of trials) and to signal-to-noise ratio: may vary across subjects
    - Are *t*-values of 4 and 100 (or *p*-values of 0.05 and 10^{-8}) really informative? The HDR of the latter is not necessarily 25 times larger than the former
  - Distributional considerations – not Gaussian
- β values
  - Have physical meaning: measure HDR magnitude = % signal change (*i.e.,* how much BOLD effect)
- Using β values ***and*** their *t*-statistics at the group level
  - More accurate approach: 3dMEMA
  - **Mostly** about the same as the conventional (β only) approach
  - Not always practical

Road Map: Choosing a program for Group Analysis?

- Starting with HDR estimated via shape-fixed method (SFM)
  - One β per condition per subject
  - It might be significantly underpowered (more later)
- Two perspectives
  - Data structure
  - Ultimate goal: list **all** the tests you want to perform
    - Possible to avoid a big model this way
    - Use a piecemeal approach with 3dttest++ or 3dMEMA
      - Perform each test on your list separately
      - Difficulty: there can be ***many*** tests you *might* want
- Most analyses can be done with **3dMVM** and **3dLME**
  - Computationally inefficient
  - Last resort: not recommended if simpler alternatives (*e.g.*, *t*-tests) are available

Road Map: Student's *t*-tests

- **3dttest++** (new version of 3dttest) and **3dMEMA**
- Not for *F*-tests except for ones with 1 DoF for numerator
  - All factors are of two levels (at most), e.g., 2 x 2, or 2 x 2 x 2
- Scenarios
  - One-, two-sample, paired
  - Univariate GLM
    - Multiple regression: 1 group + 1 or more quantitative variables
    - ANCOVA: two groups + one or more quantitative variables
    - ANOVA through dummy coding: all factors (between- or within-subject) are of **two** levels
    - AN(C)OVA: multiple between-subjects factors + one or more quantitative variables:

      https://afni.nimh.nih.gov/sscc/gangc/MEMA.html
    - One group against a constant: 3dttest/3dttest++ –singletonA
      - The "constant" can depend on voxel, or be fixed

Road Map: between-subjects ANOVA

- One-way between-subjects ANOVA
  - **3dANOVA**
  - 2 groups of subjects: 3dttest++, 3dMEMA (OK with > 2 groups too)
- Two-way between-subjects ANOVA
  - Equal #subjects across groups: **3dANOVA2** –type 1
  - Unequal #subjects across groups: **3dMVM**
  - 2 x 2 design: **3dttest++**, **3dMEMA** (OK with > 2 groups too)
- Three-way between-subjects ANOVA
  - **3dANOVA3** –type 1
  - Unequal #subjects across groups: **3dMVM**
  - 2 x 2 design: **3dttest++**, **3dMEMA** (OK with > 2 groups too)
- *N*-way between-subjects ANOVA
  - **3dMVM**

Road Map: within-subject ANOVA

- ✧ Only one group of subjects
- ✧ One-way within-subject ANOVA
  - **3dANOVA2** –type 3
  - Two conditions: **3dttest++**, **3dMEMA**
- ✧ Two-way within-subject ANOVA
  - **3dANOVA3** –type 4
    - (2 or more factors, 2 or more levels each)
  - 2 x 2 design: **3dttest++**, **3dMEMA**
- ✧ *N*-way within-subject ANOVA
  - **3dMVM**

Road Map: Mixed-type ANOVA and others

- One between- and one within-subject factor
  - Equal #subjects across groups: **3dANOVA3** –type 5
  - Unequal #subjects across groups: **3dMVM**
  - 2 x 2 design: **3dttest++**, **3dMEMA**
- More complicated scenarios
  - Multi-way ANOVA: **3dMVM**
  - Multi-way ANCOVA (between-subjects covariates only): **3dMVM**
  - HDR estimated with multiple bases: **3dANOVA3, 3dLME**, **3dMVM**
  - Missing data: **3dLME**
  - Within-subject covariates: **3dLME**
  - Subjects genetically related: **3dLME**
  - Trend analysis: **3dLME**

One-Sample Case

- One group of subjects ($n \geq 10$)
  - One condition (visual or auditory) effect
  - Linear combination of multiple effects (visual vs. auditory)
- Null hypothesis H_0: average effect = 0
  - Rejecting H_0 is of interest!
- Results
  - Average effect at group level (OLay)
  - Significance: ***t***-statistic (Thr - Two-tailed by default **in AFNI**)
- Approaches
  - **uber_ttest.py** (gen_group_command.py) – graphical interface
  - **3dttest++**
  - **3dMEMA**

One-Sample Case: Example

- **3dttest++**: taking β only for group analysis

```
3dttest++ -prefix VisGroup -mask mask+tlrc -zskip   \
 -setA 'FP+tlrc[Vrel#0_Coef]'                        \
       'FR+tlrc[Vrel#0_Coef]'                        \
       ……
       'GM+tlrc[Vrel#0_Coef]'
```

Voxel value = 0 ➔ treated it as missing

- **3dMEMA**: taking β and t-statistic for group analysis

```
3dMEMA -prefix VisGroupMEMA -mask mask+tlrc -setA Vis \
FP 'FP+tlrc[Vrel#0_Coef]' 'FP+tlrc[Vrel#0_Tstat]'     \
FR 'FR+tlrc[Vrel#0_Coef]' 'FR+tlrc[Vrel#0_Tstat]'     \
       ……
GM 'GM+tlrc[Vrel#0_Coef]' 'GM+tlrc[Vrel#0_Tstat]'     \
-missing_data 0
```

Voxel value = 0 ➔ treated it as missing

Two-Sample Case

- Two groups of subjects ($n \geq 10$ each): males and females
  - One condition (*e.g.*, visual or auditory) effect
  - Linear combination of multiple effects (*e.g.*, visual minus auditory)
  - Example: Gender difference in emotional effect of stimulus?
- Null hypothesis H_0: Group1 = Group2
  - Results
    - Group difference in average effect
    - Significance: *t*-statistic - **Two-tailed by default in AFNI**
- Approaches
  - **uber_ttest.py**, **3dttest++**, **3dMEMA**
  - One-way between-subjects ANOVA
    - **3dANOVA**: can also obtain individual group *t*-tests

Paired Case

- One groups of subjects ($n \geq 10$)
  - 2 conditions (visual or auditory): no missing data allowed (**3dLME**)
- Null hypothesis H_0: Condition1 = Condition2
  - Results
    - Average difference at group level
    - Significance: *t*-statistic (**two-tailed by default**)
- Approaches
  - **uber_ttest.py**, **gen_group_command.py, 3dttest++**, **3dMEMA**
  - One-way within-subject (repeated-measures) **ANOVA**
    - **3dANOVA2 –type 3**: can also get individual condition test
  - Missing data (**3dLME**): only 10 of 20 subjects have both βs
- Essentially same as one-sample case using contrast as input

Paired Case: Example

- **3dttest++**: comparing two conditions

```
3dttest++ -prefix Vis_Aud          \
  -mask mask+tlrc -paired -zskip          \
  -setA 'FP+tlrc[Vrel#0_Coef]'    \
        'FR+tlrc[Vrel#0_Coef]'    \
        ……
        'GM+tlrc[Vrel#0_Coef]'    \
  -setB 'FP+tlrc[Arel#0_Coef]'    \
        'FR+tlrc[Arel#0_Coef]'    \
        ……
        'GM+tlrc[Arel#0_Coef]'
```

Paired Case: Example

- 3dMEMA: comparing two conditions using subject-level response magnitudes and estimates of error levels
  - Contrast should come from each subject
    - Instead of doing contrast inside 3dMEMA itself

```
3dMEMA -prefix Vis_Aud_MEMA                                    \
-mask mask+tlrc -missing_data 0                                \
-setA Vis-Aud                                                  \
FP 'FP+tlrc[Vrel-Arel#0_Coef]' 'FP+tlrc[Vrel-Arel#0_Tstat]' \
FR 'FR+tlrc[Vrel-Arel#0_Coef]' 'FR+tlrc[Vrel-Arel#0_Tstat]' \
  ……
GM 'GM+tlrc[Vrel-Arel#0_Coef]''GM+tlrc[Vrel-Arel#0_Tstat]'
```

One-Way Between-Subjects ANOVA

- Two **or more** groups of subjects ($n \geq 10$)
  - One condition or linear combination of multiple conditions
  - Example: visual, auditory, or visual vs. auditory
- Null hypothesis H_0: Group1 = Group2
  - Results
    - Average group difference
    - Significance: ***t***- and ***F***-statistic (two-tailed by default)
- Approaches
  - **3dANOVA** (for more than 2 groups)
  - **>** 2 groups: pair-group contrasts: **3dttest++**, **3dMEMA**
  - Dummy coding: **3dttest++**, **3dMEMA** (hard work)
  - **3dMVM**

Multiple-Way Between-Subjects ANOVA

- Two or more subject-grouping factors: factorial designs
  - One condition or linear combination of multiple conditions
  - Examples: gender, control/patient, genotype, handedness
- Testing main effects, interactions, single group, group comparisons
  - Significance: ***t***- (two-tailed by default) and *F*-statistic
- Approaches
  - Factorial design (imbalance not allowed): two-way (**3dANOVA2 –type 1**), three-way (**3dANOVA3 –type 1**)
  - **3dMVM**: no limit on number of factors (imbalance OK)
  - All factors have two levels: **3dttest++**, **3dMEMA**
  - Using group coding (via covariates) with **3dttest++**, **3dMEMA**: imbalance possible

One-Way Within-Subject ANOVA

- Also called **one-way repeated-measures**: one group of subjects ($n \geq 10$)
  - Two or more conditions: extension to paired *t*-test
  - Example: happy, sad, neutral conditions
- Main effect, simple effects, contrasts, general linear tests,
  - Significance: *t*- (two-tailed by default) and F-statistic
- Approaches
  - **3dANOVA2 -type 3** (2-way ANOVA w/ 1 random factor)
  - With two conditions, **equivalent** to paired case with **3dttest++**, **3dMEMA**
  - With more than two conditions, can break into pairwise comparisons with **3dttest++**, **3dMEMA**
  - **Univariate GLM**: testing one condition is **invalid**

One-Way Within-Subject ANOVA

- Example: visual vs. auditory condition

```
3dANOVA2 -type 3 -alevels 2 -blevels 10     \
 -prefix Vis_Aud -mask mask+tlrc            \
 -amean 1 Vis -amean 2 Aud -adiff 1 2 V-A   \
  -dset 1 1  'FP+tlrc[Vrel#0_Coef]'    \
  -dset 1 2  'FR+tlrc[Vrel#0_Coef]'    \
        ......
  -dset 1 10 'GM+tlrc[Vrel#0_Coef]'    \
  -dset 2 1  'FP+tlrc[Arel#0_Coef]'    \
  -dset 2 2  'FR+tlrc[Arel#0_Coef]'    \
        ......
  -dset 2 10 'GM+tlrc[Arel#0_Coef]'
```

Two-Way Within-Subject ANOVA

- Factorial design; also known as **two-way repeated-measures**
  - 2 within-subject factors
  - Example: emotion (happy/sad) and category (visual/auditory)
- Testing main effects, interactions, simple effects, contrasts
  - Significance: *t*- (two-tailed by default) and F-statistic
- Approaches
  - **3dANOVA3 –type 4** (three-way ANOVA with one random factor)
  - All factors have 2 levels (2x2): **3dttest++**, **3dMEMA**
  - Missing data?
    - Break into *t*-tests: **3dttest++**, **3dMEMA**
    - **3dLME**

Two-Way Mixed ANOVA

- Factorial design
  - One between-subjects and one within-subject factor
  - Example: between-subject factor = gender (male and female) and within-subject factor = emotion (happy, sad, neutral)
- Testing main effects, interactions, simple effects, contrasts
  - Significance: *t*- (two-tailed by default) and *F*-statistic
- Approaches
  - **3dANOVA3 –type 5** (three-way ANOVA with one random factor)
  - If all factors have 2 levels (2x2): **3dttest++**, **3dMEMA**
  - Missing data?
    - Unequal number of subjects across groups: **3dMVM**, GroupAna
    - Break into *t*-tests: **uber_ttest.py**, **3dttest++**, **3dMEMA**
    - **3dLME**

Univariate GLM: popular in neuroimaging

- Advantages: more *flexible* than the method of Sums of Squares (SS)
  - No limit on the the number of explanatory variables (in principle)
  - Easy to handle unbalanced designs
  - Covariates can be modeled when no within-subject factors present
- Disadvantages: costs paid for the flexibility
  - Intricate dummy coding – using covariates to partition βs into subsets
  - Tedious pairing for numerator and denominator of *F*-stat
    - Can be hard to select proper denominator SS
    - Can't generalize (in practice) to any number of explanatory variables
    - Susceptible to invalid formulations and problematic post hoc tests
  - **Cannot** handle covariates in the presence of within-subject factors
  - **No** direct approach to correcting for sphericity violation
    - **Unrealistic** assumption: **same** variance-covariance structure
- **Problematic**: When residual SS is adopted for all tests
  - *F*-stat: valid only for highest order interaction of within-subject factors
  - Most post hoc tests are inappropriate/invalid

MVM Implementation in AFNI

✧ Program 3dMVM [written in R programming language]

- No tedious and error-prone dummy coding needed!
- Symbolic coding for variables and post hoc testing

Variable types

Post hoc tests

```
3dMVM  -prefix       OutputFile   -jobs 8      -SC                                  \
       -bsVars       'Grp*Age'    -wsVars      'Cond'   -qVars 'Age'                \
       -num_glt 4                                                                   \
       -gltLabel 1   Pat_Pos      -gltCode 1                  'Grp : 1*Pat Cond : 1*Pos'      \
       -gltLabel 2   Ctl_Pos-Neg  -gltCode 2                'Grp : 1*Ctl Cond : 1*Pos -1*Neg'  \
       -gltLabel 3   GrpD_Pos-Neg -gltCode 3   'Grp : 1*Ctl -1*Pat Cond : 1*Pos -1*Neg'  \
       -gltLabel 4   Pat_Age      -gltCode 4                'Grp : 1*Pat Age :'             \
       -dataTable                                                                   \
       Subj          Grp          Age          Cond     InputFile                   \
       S1            Ctl          23           Pos      S1_Pos.nii                  \
       S1            Ctl          23           Neg      S1_Neg.nii                  \
       S1            Ctl          23           Neu      S1_Neu.nii                  \
       ...                                                                          \
       S50           Pat          19           Pos      S50_Pos.nii                 \
       S50           Pat          19           Neg      S50_Neg.nii                 \
       S50           Pat          19           Neu      S50_Neu.nii
```

Data layout

Group analysis with multiple basis functions

- Fixed-Shape method (**FSM**)
- Estimead-Shape method (**ESM**) via basis functions: TENTzero, TENT, CSPLINzero, CSPLIN
  - Area under the curve (AUC) approach
    - Ignore **shape** differences between groups or conditions
    - Focus on the response **magnitude** measured by AUC
    - Potential issues: Shape information lost; Undershoot may cause trouble (canceling out some of the positive signal)
  - Better approach: maintaining shape information
    - Take individual β values to group analysis (MVM)
- Adjusted-Shape method (**ASM**) via SPMG2/3
  - Only take the major component β to group level
  - ***or***, Reconstruct HRF, and take the effect estimates (*e.g.*, AUC)

Group analysis with multiple basis functions

- Analysis with effect estimates at consecutive time grids (from TENT or CSPLIN or reconstructed HRF)
  - Used to be considered very hard to set up (in GLM)
  - Extra variable in analysis: **Time** = $t_0, t_1, \ldots, t_k$
  - One group of subjects under one condition
    - **Accurate** null hypothesis is

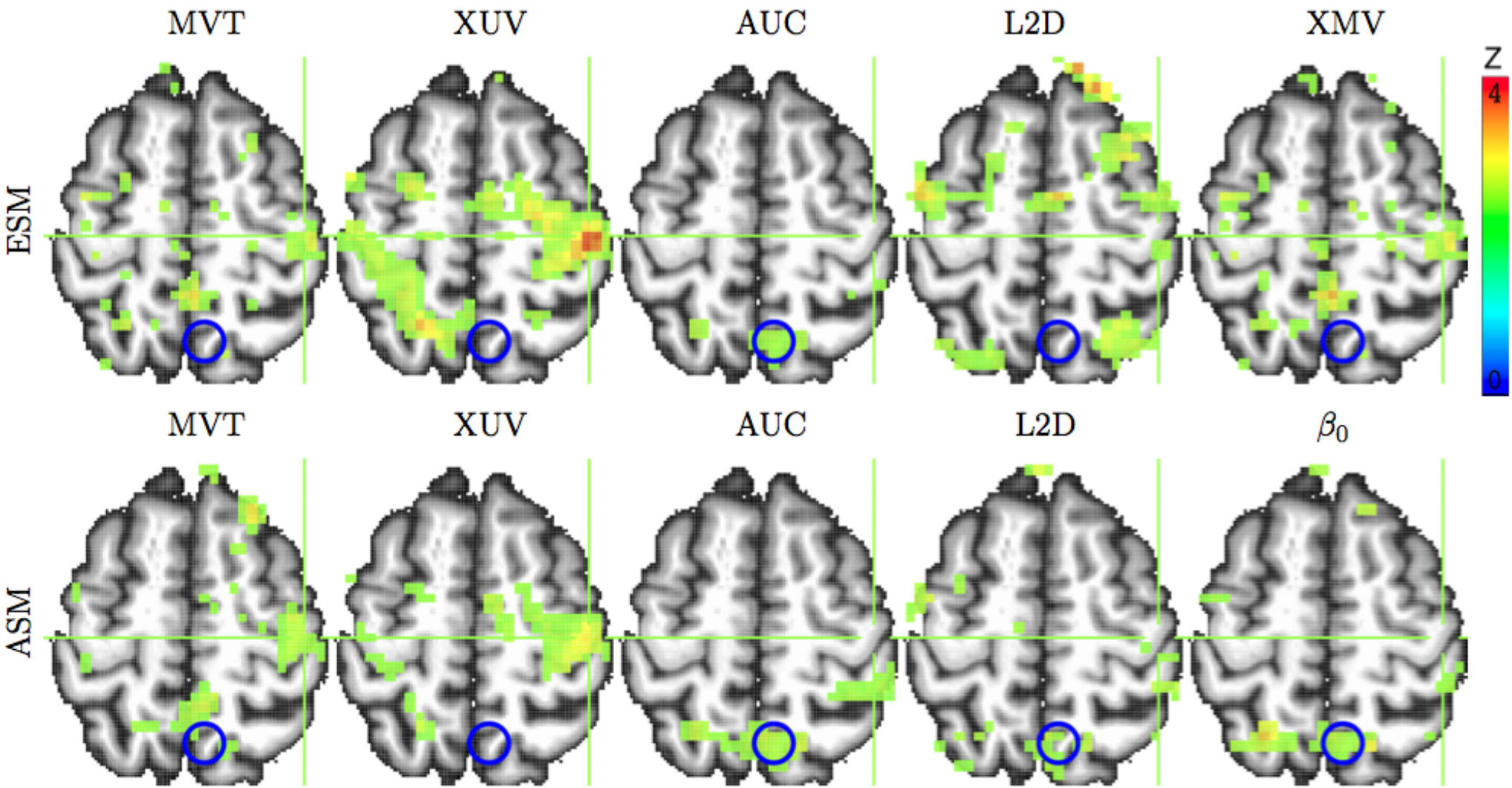
      H_0: $\beta_1=0, \beta_2=0, \ldots, \beta_k=0$ (NOT $\beta_1=\beta_2=\ldots=\beta_k$)
      - Testing the **centroid** (multivariate testing)
      - **3dLME**
    - **Approximate** hypothesis H_0: $\beta_1=\beta_2=\ldots=\beta_k$ (**main effect**)
      - **3dMVM**
    - Result: F-statistic for H_0 and t-statistic for each Time point

Group analysis with multiple basis functions

- Multiple groups (or conditions) under one condition (or group)
  - Accurate hypothesis: $\beta_1^{(1)} - \beta_1^{(2)} = 0, \beta_2^{(1)} - \beta_2^{(2)} = 0, ..., \beta_k^{(1)} - \beta_k^{(2)} = 0$
    - 2 conditions: **3dLME**
  - Approximate hypothesis: $\beta_1^{(1)} = \beta_1^{(2)}, \beta_2^{(1)} = \beta_2^{(2)}, ..., \beta_k^{(1)} = \beta_k^{(2)}$
    - Interaction
    - Multiple groups: **3dANOVA3 –type 5** (two-way mixed ANOVA: equal #subjects), or **3dMVM**
    - Multiple conditions: **3dANOVA3 –type 4**
  - Focus: do these groups/conditions have different response shape?
    - *F*-statistic for the interaction between Time and Group/Condition
    - *F*-statistic for main effect of Group: group/condition difference of AUC
    - *F*-statistic for main effect of Time: HDR effect across groups/conditions
- Other scenarios: factor, quantitative variables
  - **3dMVM**

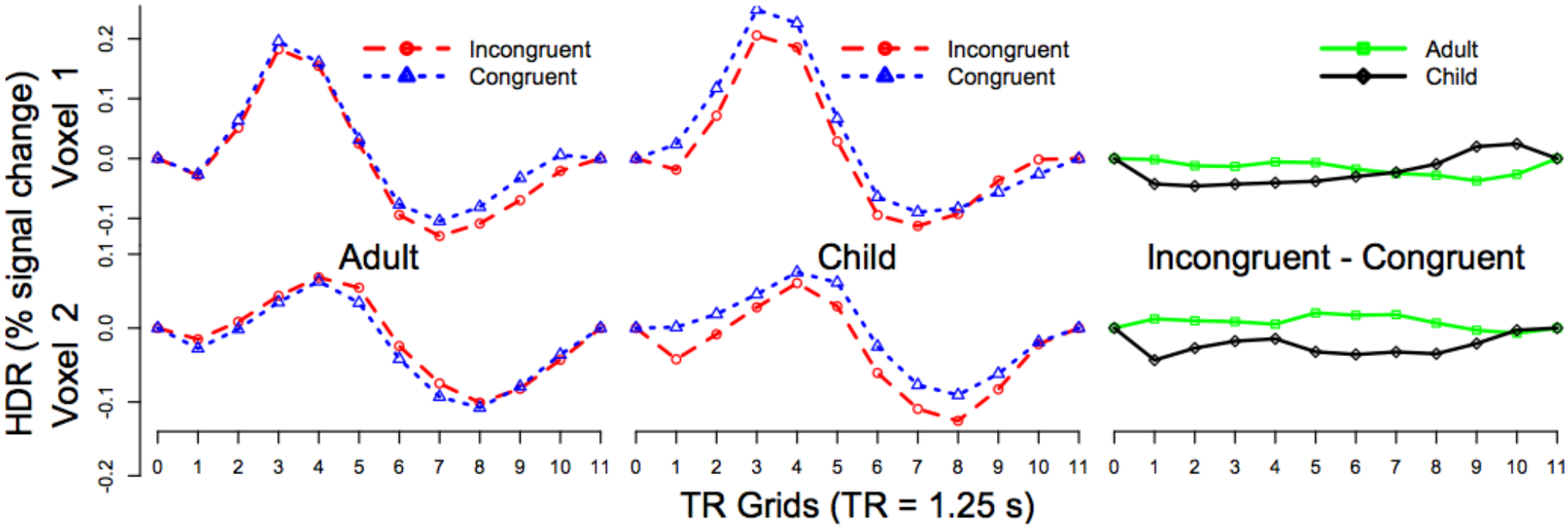
Group analysis with multiple basis functions

- 2 groups (children, adults), 2 conditions (congruent, incongruent), 1 quantitative covariate (age)
- 2 methods: HRF modeled by 10 (tents) and 3 (SPMG3) bases



Group analysis with multiple basis functions

- Advantages of ESM over FSM
  - More likely to detect HDR shape subtleties
  - Visual verification of HDR signature shape (vs. relying significance testing: *p*-values)
- Study: Adults/Children with Congruent/Incongruent stimuli (2 × 2)



Correlation analysis

- Correlation between brain response and behavioral measures

$$\hat{\beta}_i = \alpha_0 + \alpha_1 * x_i + \epsilon_i$$

  - Difference between correlation and regression?
    - Essentially the same
    - When explanatory (x_i) and response variable (β_i) are standardized (variance=1), then regression coefficient = correlation coefficient
  - Two approaches to get correlation from statistics software
    - Standardization
    - Convert *t*-statistic to *r* (or determination coefficient)

$$R^2 = t^2/(t^2 + DF)$$

    - Programs: **3dttest++**, **3dMEMA**, **3dMVM**, **3dRegAna**

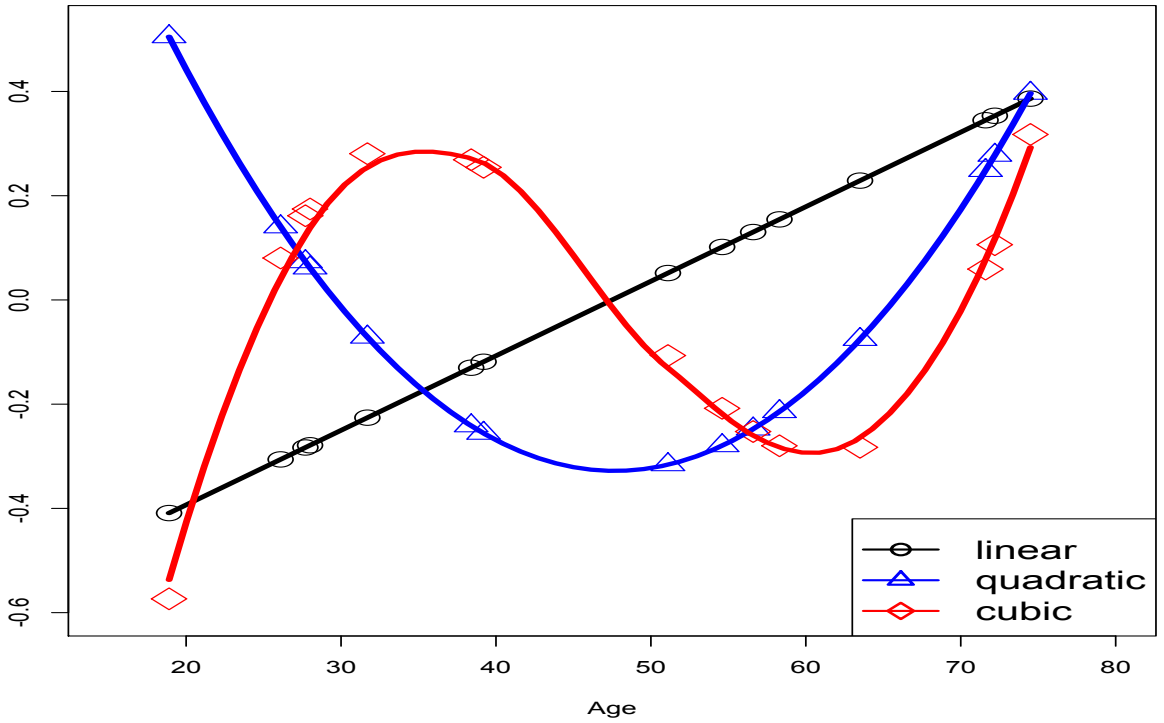
- Seed-based correlation for resting-state data
  - Fisher transform z has a variance of 1/(DoF – 2)
  - May consider further standardization by sqrt(DoF – 2)

Trend analysis

- Correlation between brain response and some gradation
  - **Linear**, **quadratic**, or **higher-order** effects
    - Habituation or attenuation effect across time (trials)
    - Between-subjects: Age, IQ
      - Fixed effect
    - Within-subject measures (covariates): morphed images
      - Random effects (trends in different subjects) : **3dLME**
  - Modeling: weights based on gradation
    - Equally-spaced: coefficients from **orthogonal polynomials**
    - With 6 equally-spaced levels, *e.g.*, 0, 20, 40, 60, 80, 100%,
      - Linear: -5 -3 -1 1 3 5
      - Quadratic: 5 -1 -4 -4 -1 5
      - Cubic: -5 7 4 -4 -7 5

Trend analysis

- Correlation between brain response and some gradation
  - Modeling: weights based on gradation
    - Not equally-spaced: constructed from, *e.g.*, poly() in R
    - Ages of 15 subjects: 31.7 38.4 51.1 72.2 27.7 71.6 74.5 56.6 54.6 18.9 28.0 26.1 58.3 39.2 63.5
    - https://afni.nimh.nih.gov/sscc/gangc/Trend.html



Trend analysis: summary

- **Cross-trials** trend: AM2 single subject analysis with weights
- Modeling with within-subject trend: **3** approaches
  - Set up GLT weights among factor levels at group level (not directly using covariate values) **3dANOVA2/3**, **3dMVM**, **3dLME**: best with equally-spaced with **even number of levels**
  - Set up the covariates as the values of a variable
    - Needs to account for deviation of each subject (random trends)
    - **3dLME**
  - Run trend analysis at individual level (*i.e.*, -gltsym), and then take the trend effect coefficient estimates to group level
    - Simpler than the other two approaches of doing trend analysis at the group level

Group analysis with quantitative variables

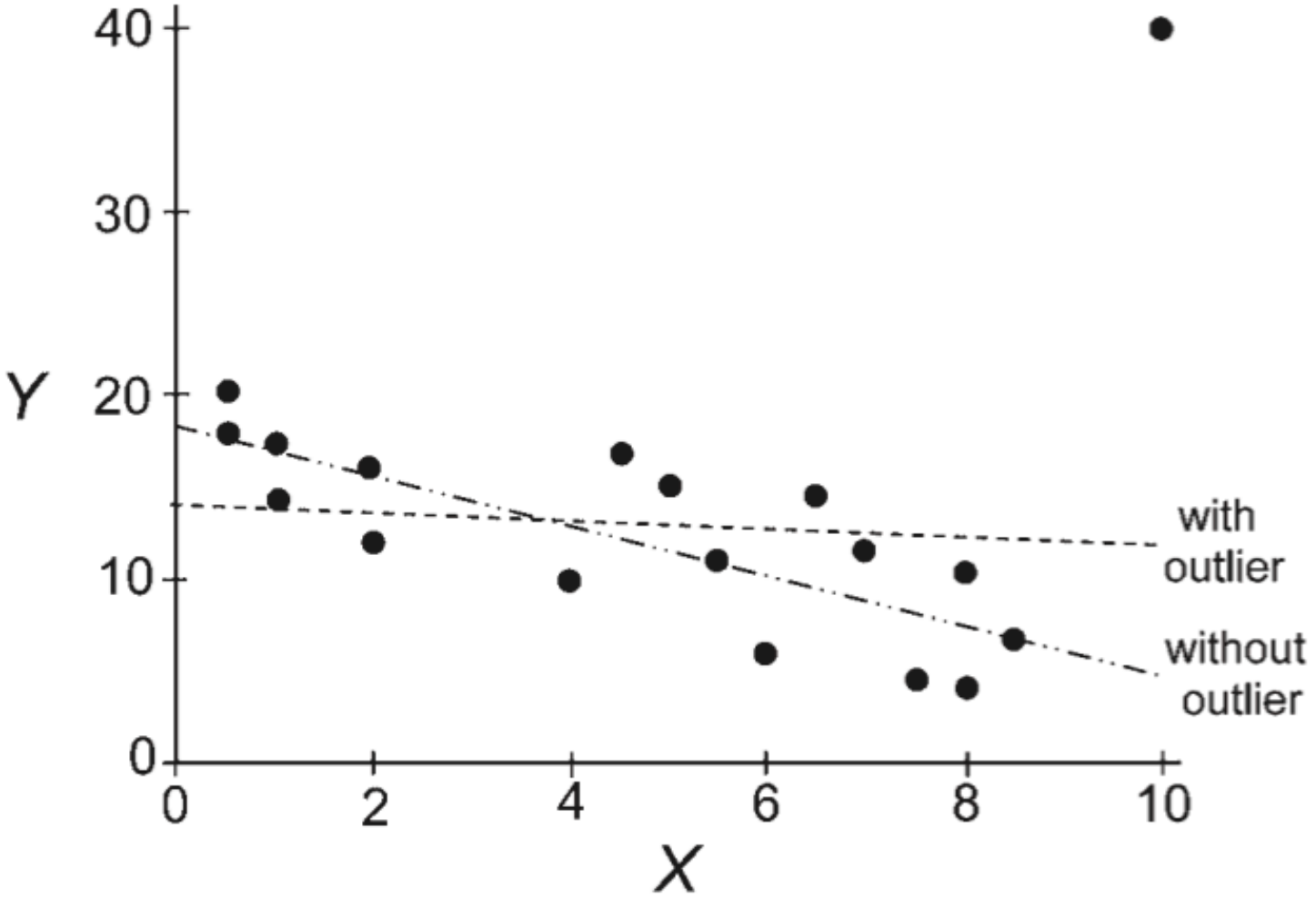
- Covariate: 3 usages
  - Quantitative (vs. categorical) variable of interest
    - Age, IQ, behavioral measures, …
  - Of no interest to the investigator (trying to remove variance)
    - Age, IQ, sex, handedness, scanner,…
  - Any explanatory variables in a model
- Variable selection
  - Infinite candidates for covariates: relying on prior information
  - Typical choices: age, IQ, RT (reaction time), …
  - RT: individual vs. group level
    - Amplitude Modulation regression: cross-trial variability at **individual** level (*cf.* Advanced Regression talk)
    - Group level: variability across subjects

Group analysis with quantitative variables

- Conventional framework
  - ANCOVA: one between-subjects factor (e.g., sex) + one quantitative variable (e.g., age)
    - Extension to ANOVA: GLM
    - Homogeneity of slopes
- Broader framework
  - Any modeling approaches involving quantitative variables
    - Regression, GLM, MVM, LME
    - Trend analysis, correlation analysis
- Interpretations
  - Regress/covariate out x?
  - "Controlling x at …", "holding x constant": centering

Caveats with covariate modeling

- Regression with few data points: sensitive to outliers
- Option -robust in 3dMVM

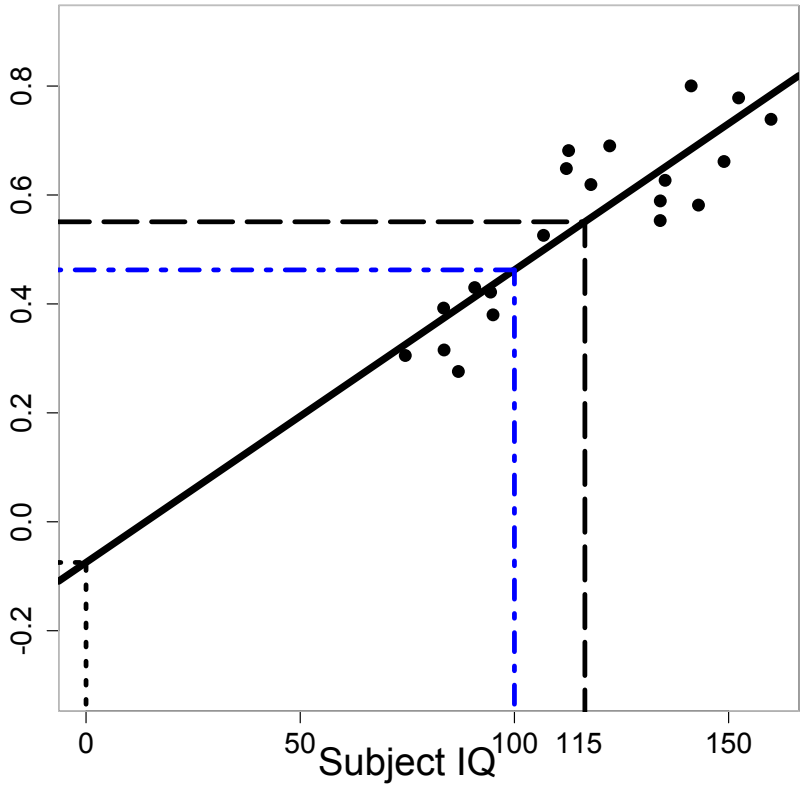


Caveats with covariate modeling

- **Specification error**: excluding a crucial explanatory variable may lead to incorrect or distorted interpretations (**spuriousness**)
  - Toddler's vocabulary ~ α * shoe size: α = .50
  - Toddler's vocabulary ~ α * shoe size + β * age: α = .04, β = .6
    - Explanatory variables (shoe size, age) are highly correlated: r = 0.8!
    - Excluding one may lead to overestimated effect for the other, but not *always* the case
- **Suppression**:
  - y (# suicide attempts) ~ 0.49 * x_1 (depression)
  - y ~ 0.19 * x_2 (amount of psychotherapy)
  - y ~ 0.70 * x_1 - 0.30 * x_2 (r_{12} = 0.7)
  - Imagine that x_1 is head motion in FMRI!

Quantitative variables: subtleties

- Regression: one group of subjects + quantitative variables $\hat{\beta}_i = \alpha_0 + \alpha_1 * x_{1i} + \alpha_2 * x_{2i} + \epsilon_i$
  - Interpretation of effects (results of regression)
    - α_1 - slope (change rate, marginal effect): effect per unit of x
    - α_0 – intercept: group effect when ***x*=0**
      - Not necessarily meaningful
      - Linearity may not hold
      - Solution: centering crucial for interpretability
      - Mean centering? or Median centering?

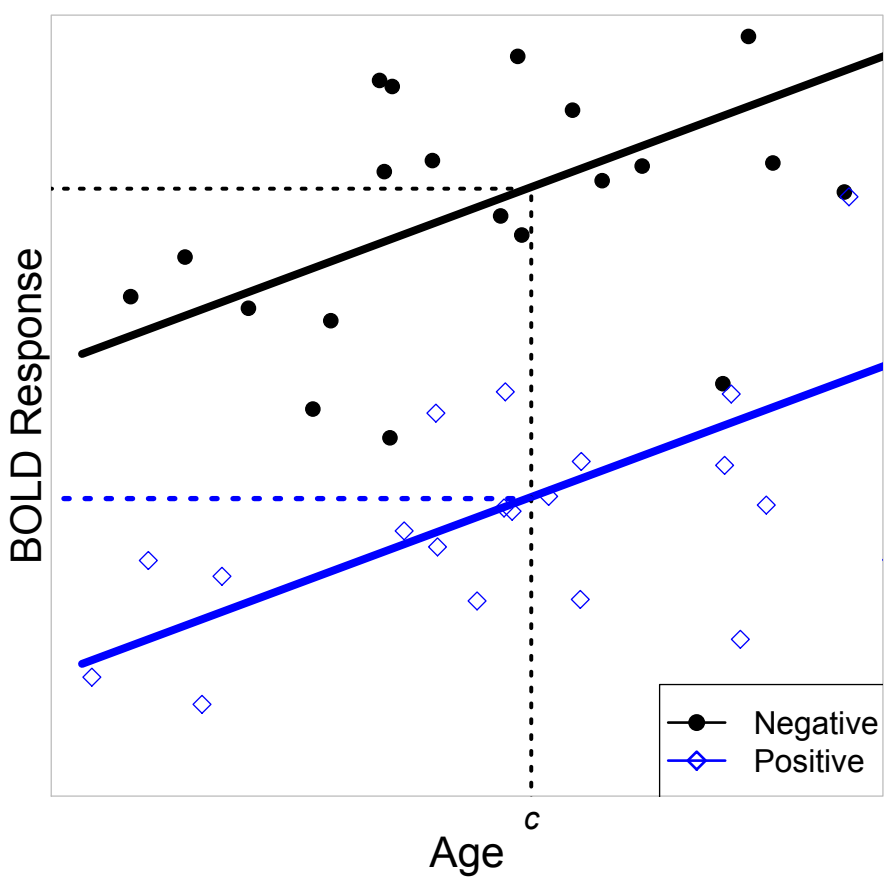


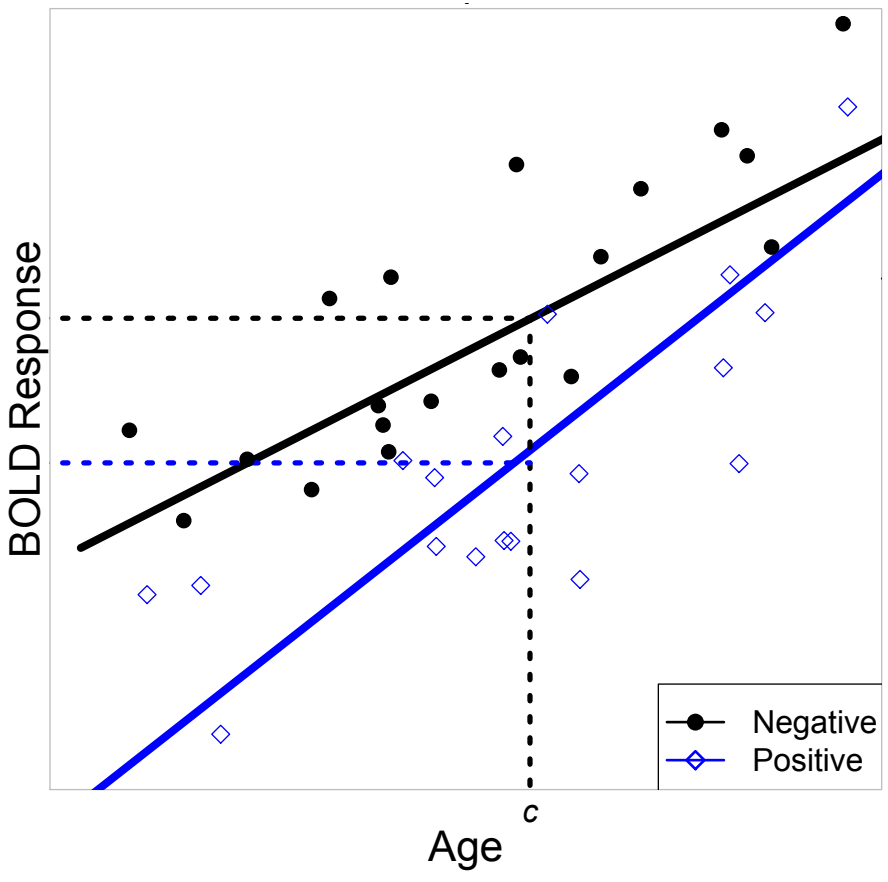
Quantitative variables: subtleties + confusion

- Trickier scenarios with two or more groups

$$\hat{\beta}_i = \alpha_0 + \alpha_1 * x_{1i} + \alpha_2 * x_{2i} + \alpha_3 * x_{3i} + \epsilon_{ij}$$

  - Interpretation of effects
    - Slope: Interaction! Same or different slope?
    - α_0 (intercept) – same or different center?

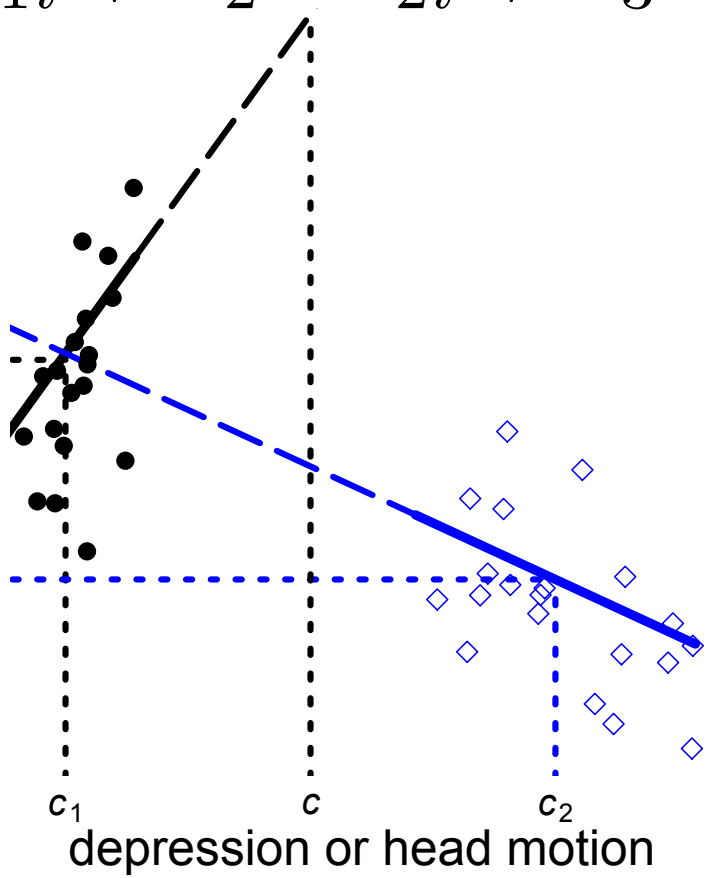




Quantitative variables: subtleties

- Trickiest scenario with two or more groups in addition to interaction

$$\hat{\beta}_i = \alpha_0 + \alpha_1 * x_{1i} + \alpha_2 * x_{2i} + \alpha_3 * x_{3i} + \epsilon_{ij}$$

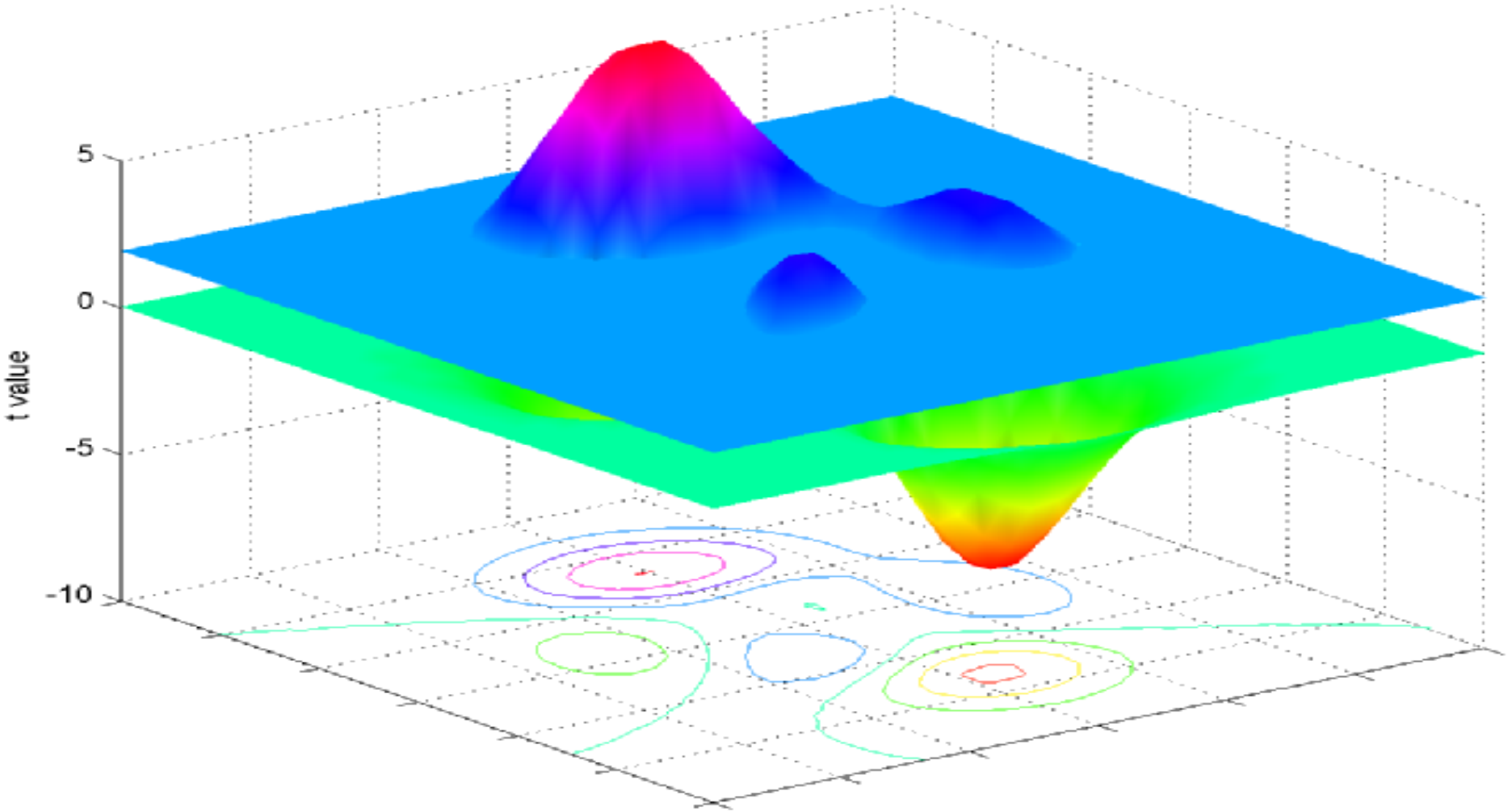


- More at **http://afni.nimh.nih.gov/sscc/gangc/centering.html**

Why should we report response magnitudes?

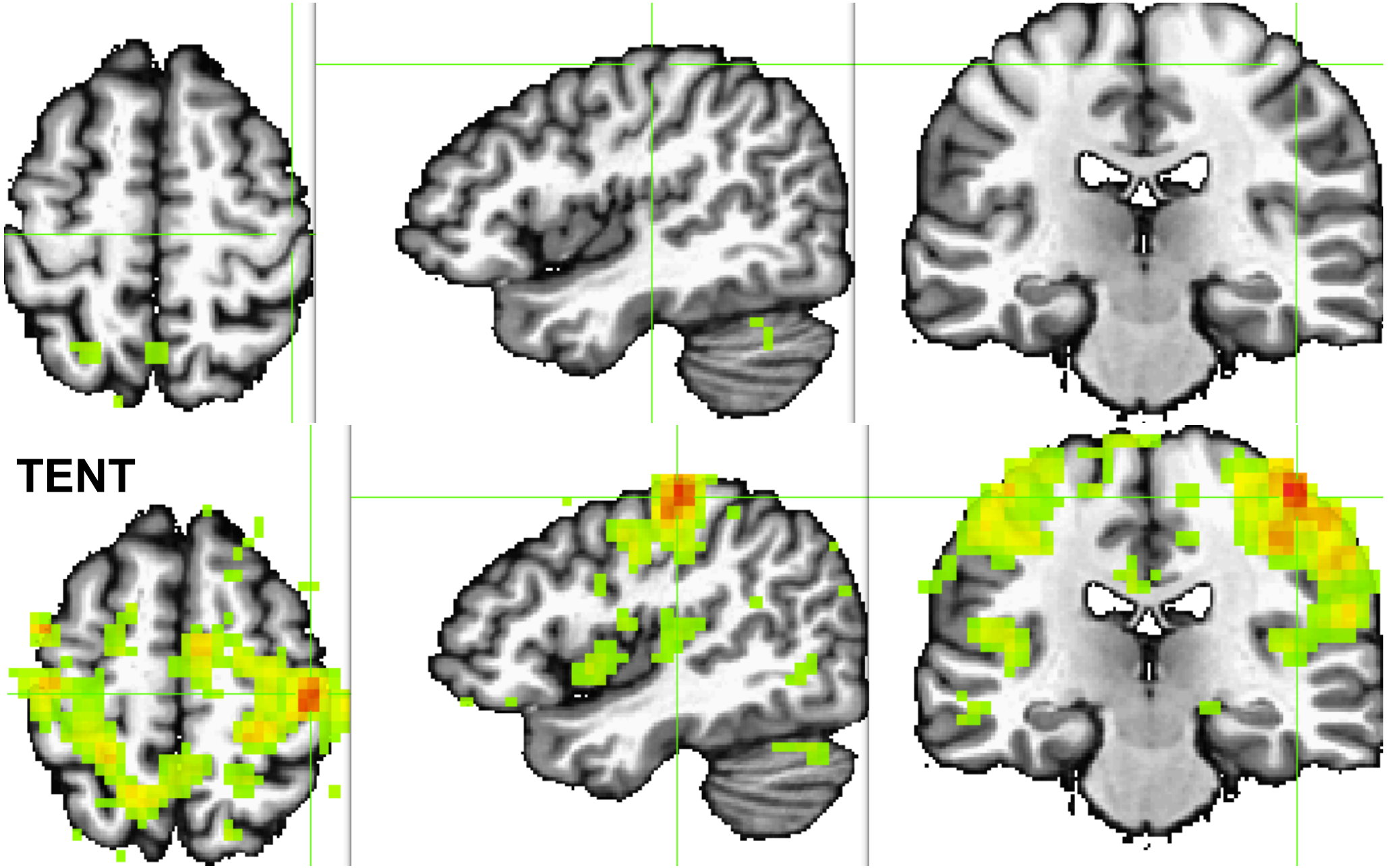
- **Unacceptable** in some fields to report only significance (peak *t* and smallest *p*)
- Neuroimaging is an exception currently!
- Obsession in FMRI about *p*-value!
  - Colored blobs of *t*-values
  - Peak voxel selected based on peak *t*-value
- Science is about reproducibility
  - Response amplitude should be of primacy focus
  - Statistics are only for thresholding
    - No physical dimension, and are a mix of response size and noise magnitude
    - Once surviving threshold, specific values are not informative

- **Basics: Null hypothesis significance testing (NHST)**
  - Should science be based on a binary (Yes/No) inference?
    - If a cluster fails to survive thresholding, it has no value?
    - Small Volume Correction (SVC): Band-Aid solution

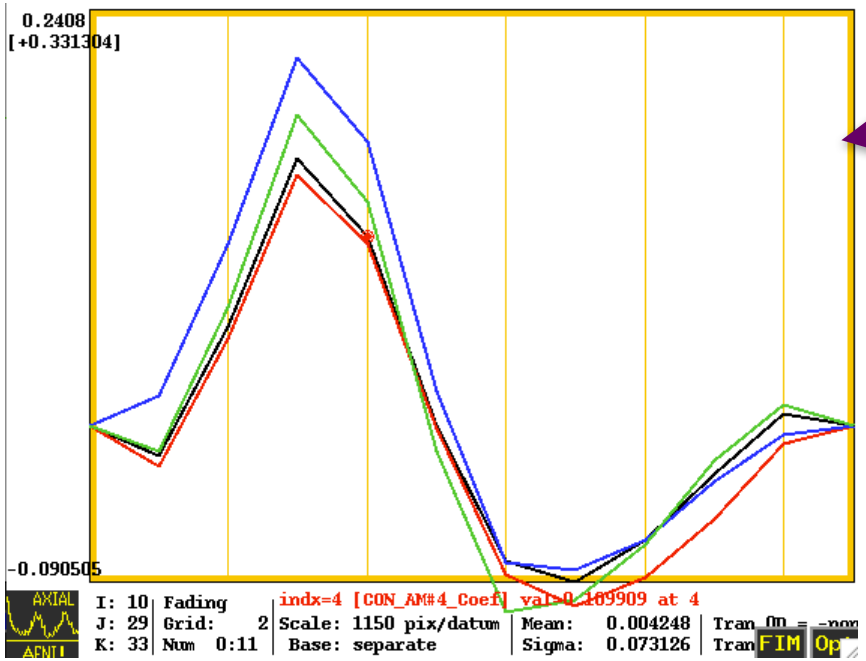


Modeling strategy & results: an example

SPMG3: 1st β (canonical HDR) [voxel-wise p=0.01]

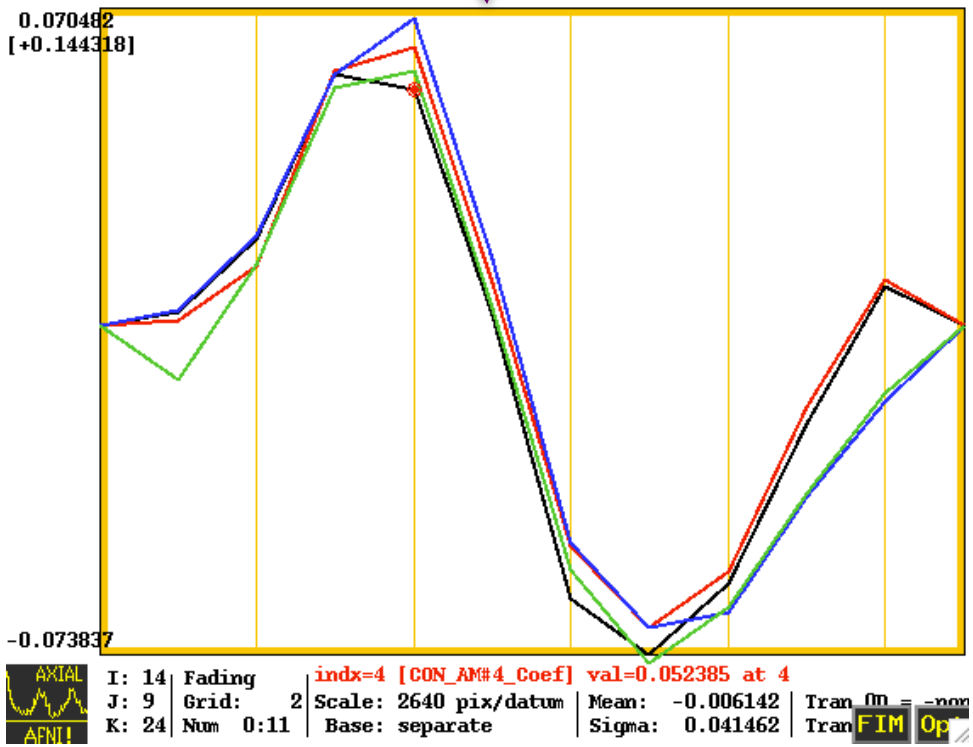


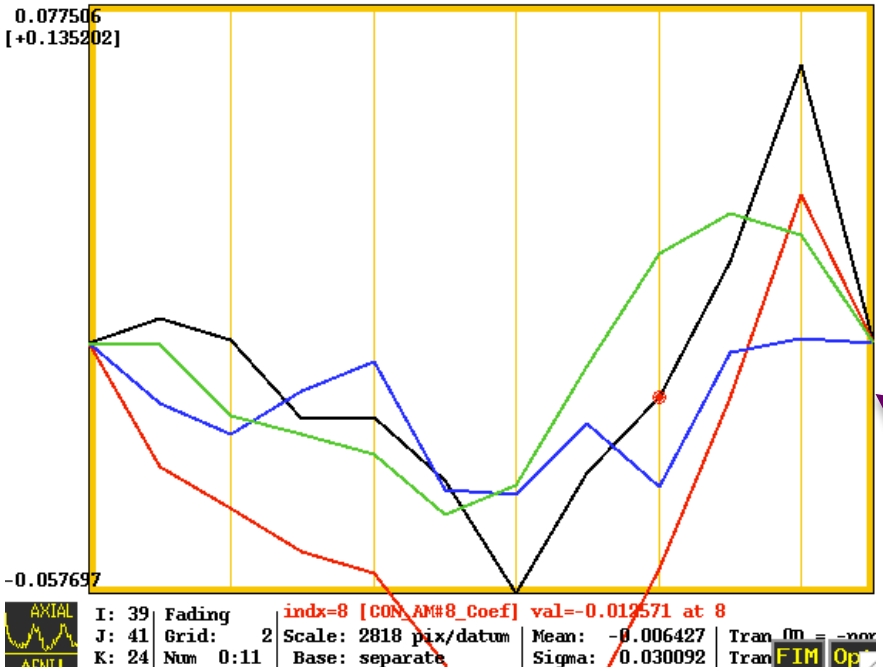
Is *p*-value everything? An example



$p=10^{-7}$

$p=0.5$





$p=10^{-4}$

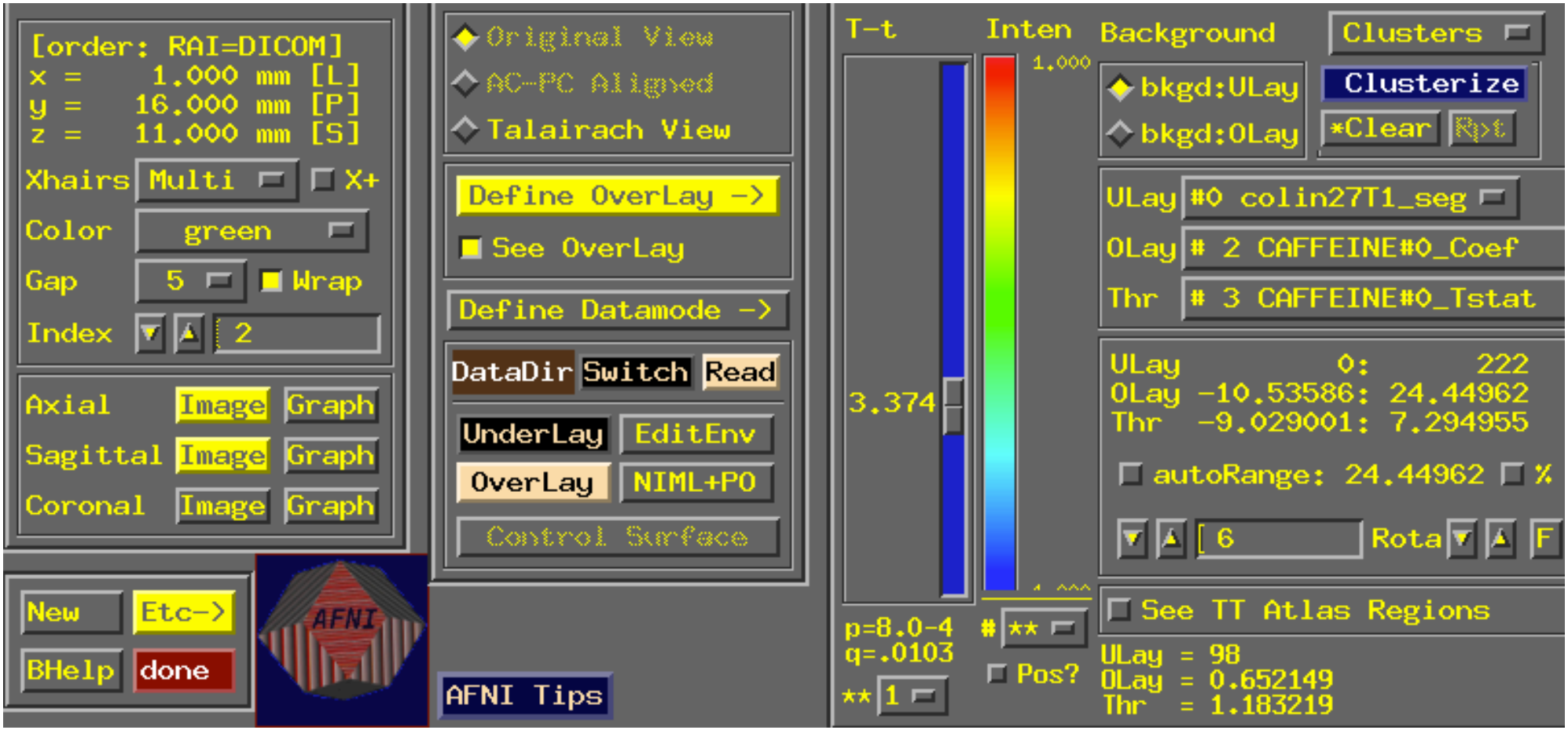
Advantages of ESM

- Multiple basis functions
  - TENTzero, TENT, CSPLINzero, CSPLIN
  - Similar to FIR in SPM, but FIR does not allow non-TR-synchronized modeling
- Higher statistical power than FSM and ASM
  - More likely to identify activations
- Extra support for true positives (TP) with HRF signature shape
  - Unavailable from FFM and ASM
- Crucial evidence if significance is marginal: false negatives (FP)
- Avoiding false positives (FP)
- Works best for event-related experiments
  - Useful for block designs if concerned about habituation, attenuation,…

How rigorous about corrections?

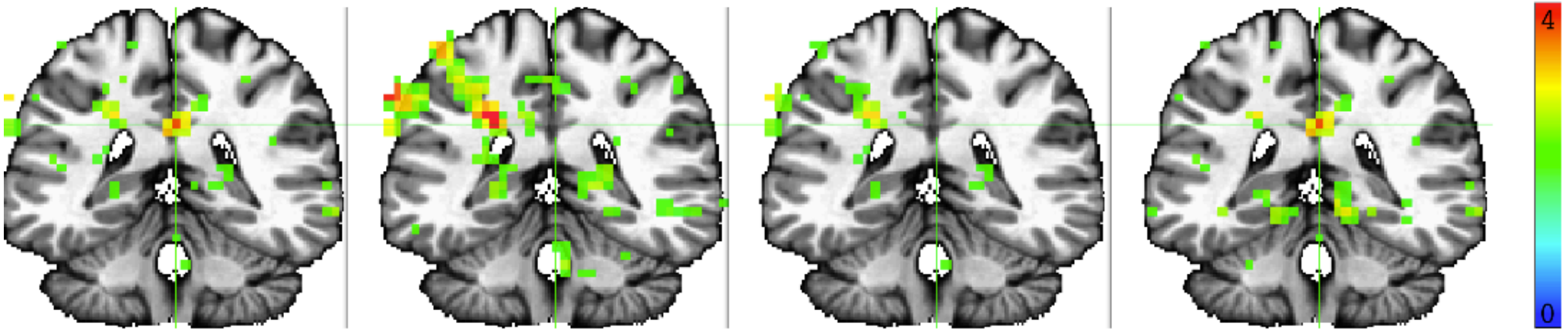
- Two types of correction
  - Multiple testing correction n(MTC): **same** test across brain
    - FWE, FDR, SVC(?)
    - People (esp. reviewers) worship this!
  - Multiple comparisons correction (MCC): **different** tests
    - Happy vs. Sad, Happy vs. Neutral, Sad vs. Neutral
    - Two one-sided *t*-tests: *p*-value is ½ of two-sided test!
    - How far do you want to go?
      - Tests in one study
      - Tests in all FMRI or all scientific studies?
    - Nobody cares about this issue in FMRI (for unknown reasons)
- Many reasons for correction failure (loss of statistical significance)
  - Region size, number of subjects, alignment quality, substantial cross-subject variability (anxiety disorder, depression, …)

Presenting response magnitudes



Presenting response magnitudes

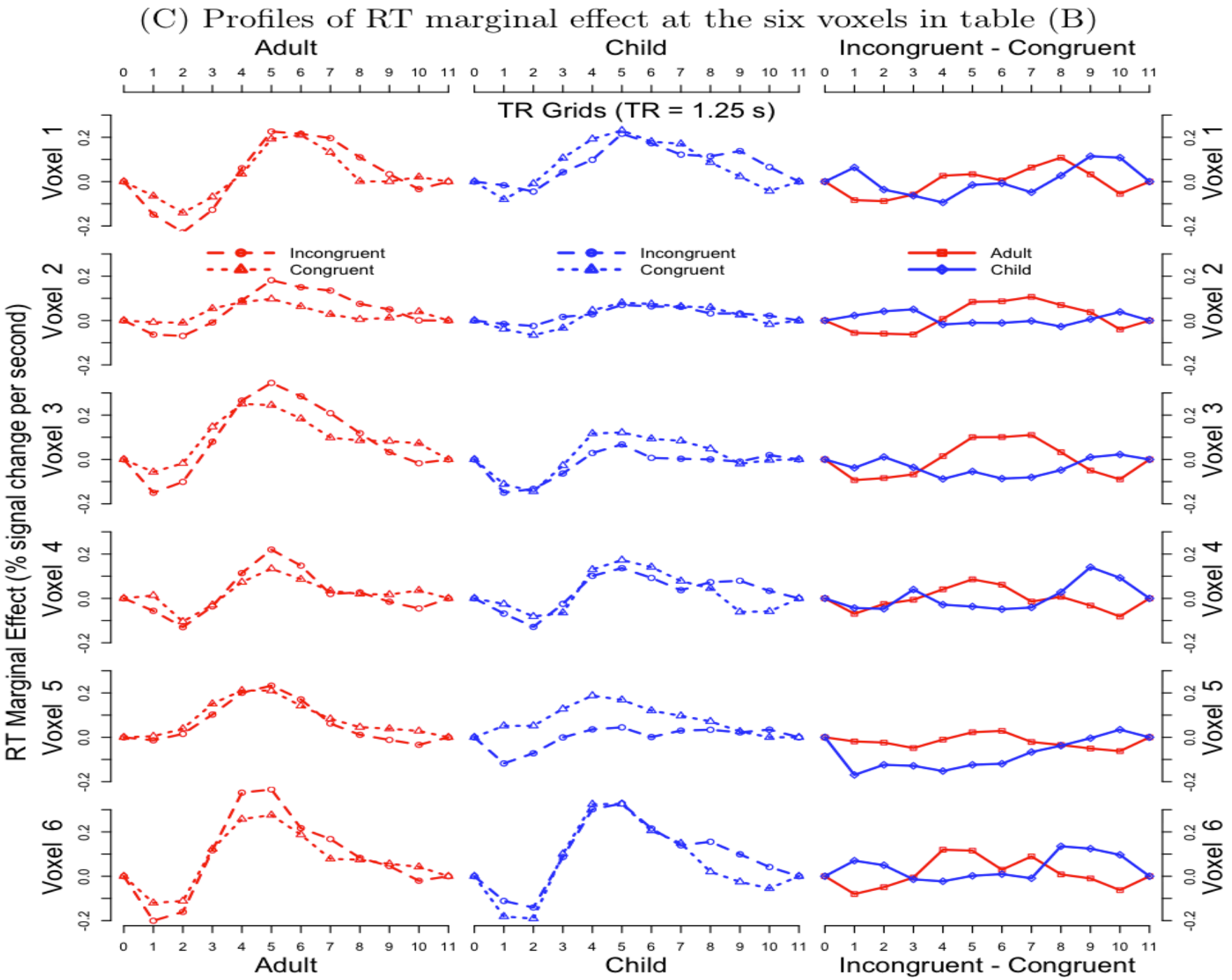
(A) Coronal view of interaction effect of Group:Condition:Time



(B) Sphericity scenarios at six representative voxels

Voxel		Sphericity			**UVT-UC**	**UVT-SC**	**MVT-WS**	**HT**
No.	coordinates	Mauchly p-value	ϵ_{GG}	ϵ_{HF}	p-value	p-value	p-value	taking
1	-2 36 27	0	0.32	0.35	0.28	0.31	0.00021	MVT-WS
2	-33 -5 42	0	0.42	0.46	3.8×10^{-6}	8.4×10^{-4}	1.6×10^{-4}	MVT-WS
3	-50 -16 24	0	0.45	0.50	1.6×10^{-4}	0.0041	0.14	MVT-WS
4	-5 -20 23	8.7×10^{-6}	0.68	0.79	1.8×10^{-5}	0.0001	0.008	UVT-SC
5	37 68 20	0	0.30	0.32	0.012	0.074	0.15	MVT-WS
6	-36 -16 7	0	0.53	0.60	1.8×10^{-5}	5.3×10^{-4}	0.0019	UVT-SC

Presenting response magnitudes



IntraClass Correlation (ICC)

- Reliability (consistency, agreement/reproducibility) across two or more measurements of the same condition/task (sessions, scanners, sites, studies, twins -- monozygous or dizygous): extent to which the levels of a factor are related to each other
  - Example: 20 subjects scanned in two scanners (effect estimate of a condition/task, contrast between 2 conditions/tasks, functionality measure, etc.)
  - Classic example in Shrout and Fleiss (1979): n targets are rated by k raters/judges
  - Relationship with Pearson correlation:
    - ICC is the Pearson correlation between any two measurements
  - Difference with Pearson correlation
    - Pearson correlation can be for any two different types of measure: e.g., BOLD response vs. RT
    - ICC is for the **same** measurement with the **same** assumption $G(\mu, \sigma^2)$

IntraClass Correlation (ICC)

- Three different definitions
  - One-way random-effects ANOVA

$$x_{ij} = \mu + r_i + w_{ij}$$
where $i = 1, \ldots, n$ and
$j = 1, \ldots, k$.

  - Assumptions: subject $r_i \sim G(0, \sigma_r^2)$, $w_{ij} \sim G(0, \sigma_w^2)$
  - Order cannot be assigned across multiple measurements
    - *e.g.*, twins: fixed or random effect of twins (index *j*) not considered
  - **ICC(1,1)** in Shrout & Fleis (1979)

$$\frac{\sigma_r^2}{\sigma_r^2 + \sigma_w^2}$$

  - Conceptualized as an LME model

IntraClass Correlation (ICC)

- Three different definitions
  - Two-way random-effects ANOVA

$$x_{ij} = \mu + r_i + c_j + e_{ij}$$

where $i = 1, \ldots, n$ and $j = 1, \ldots, k$.

  - Assumptions: subject $r_i \sim G(0, \sigma_r^2)$, session $c_j \sim G(0, \sigma_c^2)$, $w_{ij} \sim G(0, \sigma_w^2)$
  - Order can be assigned across multiple measurements
    - *e.g.*, session: random effect (index j) – no systematic difference across sessions
  - **ICC(2,1)** in Shrout & Fleis (1979)

$$\frac{\sigma_r^2}{\sigma_r^2 + \sigma_c^2 + \sigma_e^2}$$

  - Conceptualized as an LME model

IntraClass Correlation (ICC)

- Three different definitions
  - Two-way mixed-effects ANOVA

$$x_{ij} = \mu + r_i + c_j + e_{ij}$$

where $i = 1, \ldots, n$ and $j = 1, \ldots, k$.

  - Assumptions: subject $r_i \sim G(0, \sigma_r^2)$, $w_{ij} \sim G(0, \sigma_e^2)$
  - Order can be assigned across multiple measurements
    - *e.g.*, scanner: fixed effect (index j) – systematic difference across scanners
  - **ICC(3,1)** in Shrout & Fleis (1979) $\dfrac{\sigma_r^2}{\sigma_r^2 + \sigma_e^2}$
  - Conceptualized as an LME model

IntraClass Correlation (ICC)

- Three different definitions
  - Pearson correlation
  - Proportion of total variance that can be accounted for across-subject variance
  - ICC(1,1) and ICC(2,1): **agreement/reproducibility**;

    ICC(3,1): **consistency**
- Implemented in **3dLME –ICC** in AFNI
  - The 3 types of ICC can be specified through options –model and –ranEff
  - ICC of interest: cross-subjects ICC (labeled with subject)
- Will be migrated to **3dICC**

IntraClass Correlation (ICC)

- Three different definitions
- Implemented in **3dLME –ICC** in AFNI
- What is the advantage of LME over ANOVA?
  - ANOVA may render negative ICC values: not interpretable nor meaningful
  - LME places a boundary at 0, so all ICC estimates are ≥0
  - Huge flexibility of LME: easy to incorporate fixed- and random-effects in the model (e.g., age, RT, etc.)
- Problem with zero ICC
  - Stuck at a numerical bounardy
  - Not practical meaningful
- Bayesian approach: a tiny nudge by a weak prior
  - Implemented in **3dLME –ICCb** in AFNI (**recommended**)

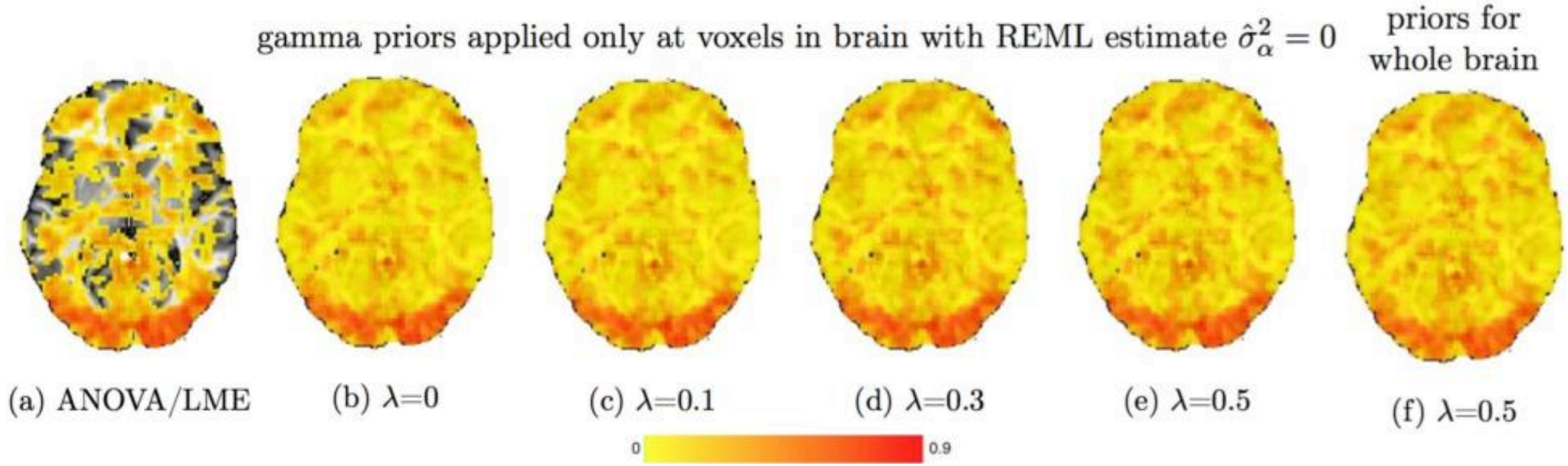
IntraClass Correlation (ICC)

- Three different definitions
- Implemented in **3dLME –ICCb** in AFNI (**recommended**)
- Prior: Gamma density function

$$h(\sigma;\theta,\lambda) = \frac{\lambda^{\theta}}{\Gamma(\theta)}\sigma^{\theta-1}e^{-\lambda\sigma}$$

$$\theta > 0, \lambda > 0$$

- Performance of Bayesian approach



IntraClass Correlation (ICC)

- Three different definitions
- Implemented in **3dLME –ICCb** in AFNI (**recommended**)
- Future developments?
  - A standalone program **3dICC**
  - Significance testing for ICC
  - Incorporation of effect estimate reliability into the model

Group Analysis: Non-Parametric Approach

- Parametric approach
  - When have enough number subjects: $n > 10$
  - Random effects of subjects: usually Gaussian distribution
  - Individual and group analyses: separate

- Non-parametric approach
  - Moderate number of subjects: $4 < n < 10$
  - No assumption of data distribution (e.g., normality)
  - Statistics based on ranking or permutation
  - Individual and group analyses: separate

Non-Parametric Analysis

- Ranking-based: roughly equivalent to permutation tests
  - **3dWilcoxon** (~ paired *t*-test)
  - **3dFriedman** (~ one-way within-subject with **3dANOVA2**)
  - **3dMannWhitney** (~ two-sample *t*-test)
  - **3dKruskalWallis** (~ between-subjects with **3dANOVA**)
- Pros: Less sensitive to outliers (more robust)
- Cons
  - Multiple testing correction **limited** to FDR (**3dFDR**)
  - Less flexible than parametric tests
    - Can't handle complicated designs with more than one fixed-effects factor
    - Can't handle covariates
- Direct permutation approach?

Group Analysis: Fixed-Effects Analysis (very old)

- When to consider?
  - LME approach
  - Group level: a few subjects: $n < 6$
  - Individual level: combining multiple runs/sessions
- Case study: difficult to generalize to whole population
- Model $\beta_i = b + \varepsilon_i$, $\varepsilon_i \sim N(0, \sigma_i^2)$, σ_i^2: within-subject variability
  - Fixed in the sense that cross-subject variability is not considered
- Direct fixed-effects analysis (**`3dDeconvolve/3dREMLfit`**)
  - Combine data from all subjects and then run regression
- Fixed-effects meta-analysis (**`3dcalc`**) : weighted least squares
  - $\beta = \sum w_i \beta_i / \sum w_i$, $w_i = t_i / \beta_i$ = weight for ith subject
  - $t = \beta \sqrt{\sum w_i}$

Group Analysis Program List

- **3dttest++** (one-sample, two-sample and paired *t*) + covariates (voxel-wise is allowed, *e.g.*, GM fraction)
- **3dMEMA** (R package for mixed-effects analysis, *t*-tests plus covariates)
- **3ddot** (correlation between two datasets)
- **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)
- **3dMVM** (AN(C)OVA, and within-subject MAN(C)OVA)
- **3dLME** (R package for sophisticated cases)
- **3dttest** (obsolete: one-sample, two-sample and paired *t*)
- **3dRegAna** (obsolete: regression/correlation, covariates)
- **GroupAna** (mostly obsolete: Matlab package for up to four-way ANOVA)

FMRI Group Analysis Comparison

		AFNI	SPM	FSL
t-test (one-, two-sample, paired)		3dttest++, 3dMEMA	Yes	FLAME1, FLAME1+2
One categorical variable: one-way ANOVA		3dANOVA/2/3, GroupAna	Only **one** WS factor: full and flexible factorial design	Only **one** within-subject factor: GLM in FEAT
Multi-way AN(C)OVA		3dANOVA2/3, GroupAna, 3dMVM	---	---
Between-subject covariate		3dttest++, 3dMEMA, 3dMVM	Partially	Partially
Sophisticated situations	Covariate + within-subject factor	3dLME	---	---
	Subject adjustment in trend analysis			
	Basis functions			
	Missing data			

ISC: Overview

- Naturalistic FMRI
  - A middle point between task-related and resting-state scanning
  - A special case of task-related FMRI: task from beginning to end
  - Resting-state data: an asymptotic case of naturalistic canning
- Challenges of analyzing naturalistic scanning data
- Survey of previous approaches
- Exploration of new nonparametric methods
- Flexibility of linear mixed-effects (LME) modeling (**program publicly available**)
- Potential application to resting-state data
  - Focus on whole brain instead of one seed

Two popular types of FMRI scanning

- Task-related experiments
  - Meticulously designed, well controlled
  - Event-related or block design
  - Effect of interest: regional responses to a task or a contrast
  - Models: responses estimated through time series regression
  - Potential issues
    - Artificial tasks: absence of distinctive textures of real life events
    - Artificial or discrete intervals between trials
    - Poor understanding/modeling, low sensitivity (underpowered)

- Resting state
  - No explicit tasks
  - Spontaneous, intrinsic fluctuations
  - Effect of interest: regional correlation, networks
  - Models: seed-based correlation, data-driven methods, etc.
  - Caveats: difficult to separate physiological confounds, arbitrary in data manipulations/interpretations

Naturalistic scanning

- Subjects view a natural scene during scanning
  - Visuoauditory movie clip (e.g., http://studyforrest.org/)
  - Neural responses shared across languages
  - Music, speech, games, …

- Duration: lasting for a few minutes or more
- Close to naturalistic settings: minimally manipulated; naturalistically, continuously, and dynamically evolving
- Effect of interest
  - Extent of synchronization/entrainment, similarity, or shared processing at the same brain regions across subjects in shared memory, communication and understanding through a common ground

- Hasson et al., 2004. Intersubject synchronization of cortical activity during natural vision. Science 303:1634-1640.

Inter-Subject Correlation (ISC)

- Modeling with task-related regressors won't work
  - One regressor for the whole task: BOLD can't be separated from baseline and drift effects
  - Feature extractions: too rich or complicated to be practical

- Inter-subject correlation (ISC)
  - Proper preprocessing
    - Nonlinear alignment to template space
    - Removing physiological confounds (e.g., regressing out signal in the white matter and principal components from the CSF signal)
    - Censoring out time points when significant motion occurred

Inter-Subject Correlation (ISC)

- Inter-subject correlation (ISC)
  - Correlation of time series between two subjects at the same voxel
  - No presumption of HDR
  - Measuring synchronization/similarity/entrainment
  - Avoiding the arbitrariness of seed selection

- Voxel-wise ISC between any subject pair
  - n = 3 subjects (A, B, C): 3 ISC values (AB, AC, BC)
  - n = 4 subjects: 6 ISCs
  - n = 5 subjects: 10 ISCs
  - n subjects: $n(n\text{-}1)/2$ ISCs

- ISC group analysis
  - Summarization at the group level
  - Investigate differences across groups in synchronization (ISC)
  - Difficulty: some of ISC values are correlated

    n independent samples correspond to $n(n\text{-}1)/2$ ISCs

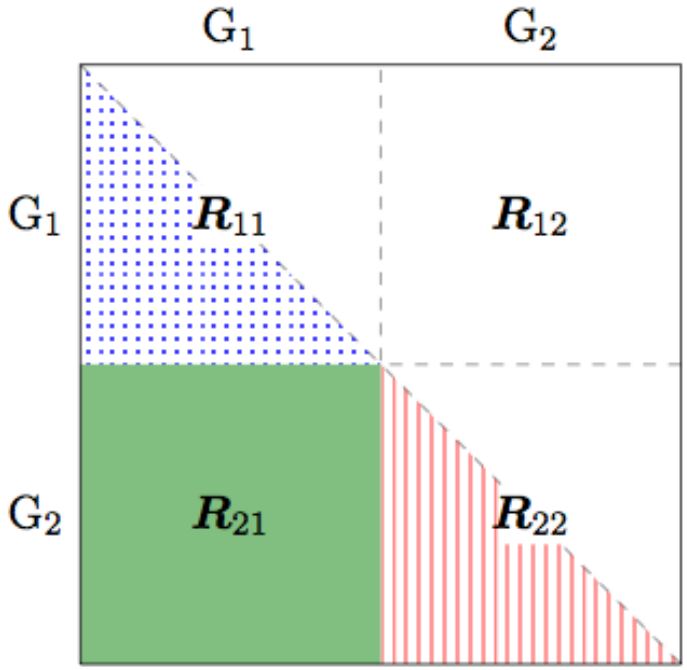
ISC group analysis

- Voxel-wise ISC matrix (usually Fisher-transformed)
  - One group

$$\boldsymbol{R}^{(n)} = \begin{array}{c} \\ S_1 \\ S_2 \\ S_3 \\ \vdots \\ S_n \end{array} \begin{array}{c} \begin{array}{ccccc} S_1 & S_2 & S_3 & \cdots & S_n \end{array} \\ \begin{pmatrix} 1 & r_{12} & r_{13} & \cdots & r_{1n} \\ r_{21} & 1 & r_{23} & \cdots & r_{2n} \\ r_{31} & r_{32} & 1 & \cdots & r_{3n} \\ \vdots & \vdots & \vdots & \ddots & \vdots \\ r_{n1} & r_{n2} & r_{n3} & \cdots & 1 \end{pmatrix} \end{array}$$

$$\boldsymbol{Z}^{(n)} = \begin{array}{c} \\ S_1 \\ S_2 \\ S_3 \\ \vdots \\ S_n \end{array} \begin{array}{c} \begin{array}{ccccc} S_1 & S_2 & S_3 & \cdots & S_n \end{array} \\ \begin{pmatrix} - & z_{12} & z_{13} & \cdots & z_{1n} \\ z_{21} & - & z_{23} & \cdots & z_{2n} \\ z_{31} & z_{32} & - & \cdots & z_{3n} \\ \vdots & \vdots & \vdots & \ddots & \vdots \\ z_{n1} & z_{n2} & z_{n3} & \cdots & - \end{pmatrix} \end{array}$$

  - Two groups
    - Within-group ISC: R11, R22
    - Inter-group ISC: R21
    - 3 group comparisons: R11 vs R22, R11 vs R21, R22 vs R21



Correlation pattern of ISC values

- 2 ISC values associated with a common subject are correlated with each other: 5 subjects, 10 ISC values
- $\rho \neq 0$ characterizes non-independent relationship

	Z_{21}	Z_{31}	Z_{41}	Z_{51}	Z_{32}	Z_{42}	Z_{52}	Z_{43}	Z_{53}	Z_{54}
Z_{21}	1	ρ	ρ	ρ	ρ	ρ	ρ	0	0	0
Z_{31}	ρ	1	ρ	ρ	ρ	0	0	ρ	ρ	0
Z_{41}	ρ	ρ	1	ρ	0	ρ	0	ρ	0	ρ
Z_{51}	ρ	ρ	ρ	1	0	0	ρ	0	ρ	ρ
Z_{32}	ρ	ρ	0	0	1	ρ	ρ	ρ	ρ	0
Z_{42}	ρ	0	ρ	0	ρ	1	ρ	ρ	0	ρ
Z_{52}	ρ	0	0	ρ	ρ	ρ	1	0	ρ	ρ
Z_{43}	0	ρ	ρ	0	ρ	ρ	0	1	ρ	ρ
Z_{53}	0	ρ	0	ρ	ρ	0	ρ	ρ	1	ρ
Z_{54}	0	0	ρ	ρ	0	ρ	ρ	ρ	ρ	1

- **Challenge**: how to handle this irregular correlation matrix?

ISC group analysis: previous methods

- Student's *t*-test
  - Independence violation acknowledged but not accounted for
  - Justification via observations that "null data" (generated by ISC values with randomly shifted time series) followed *t*(*N*-1)

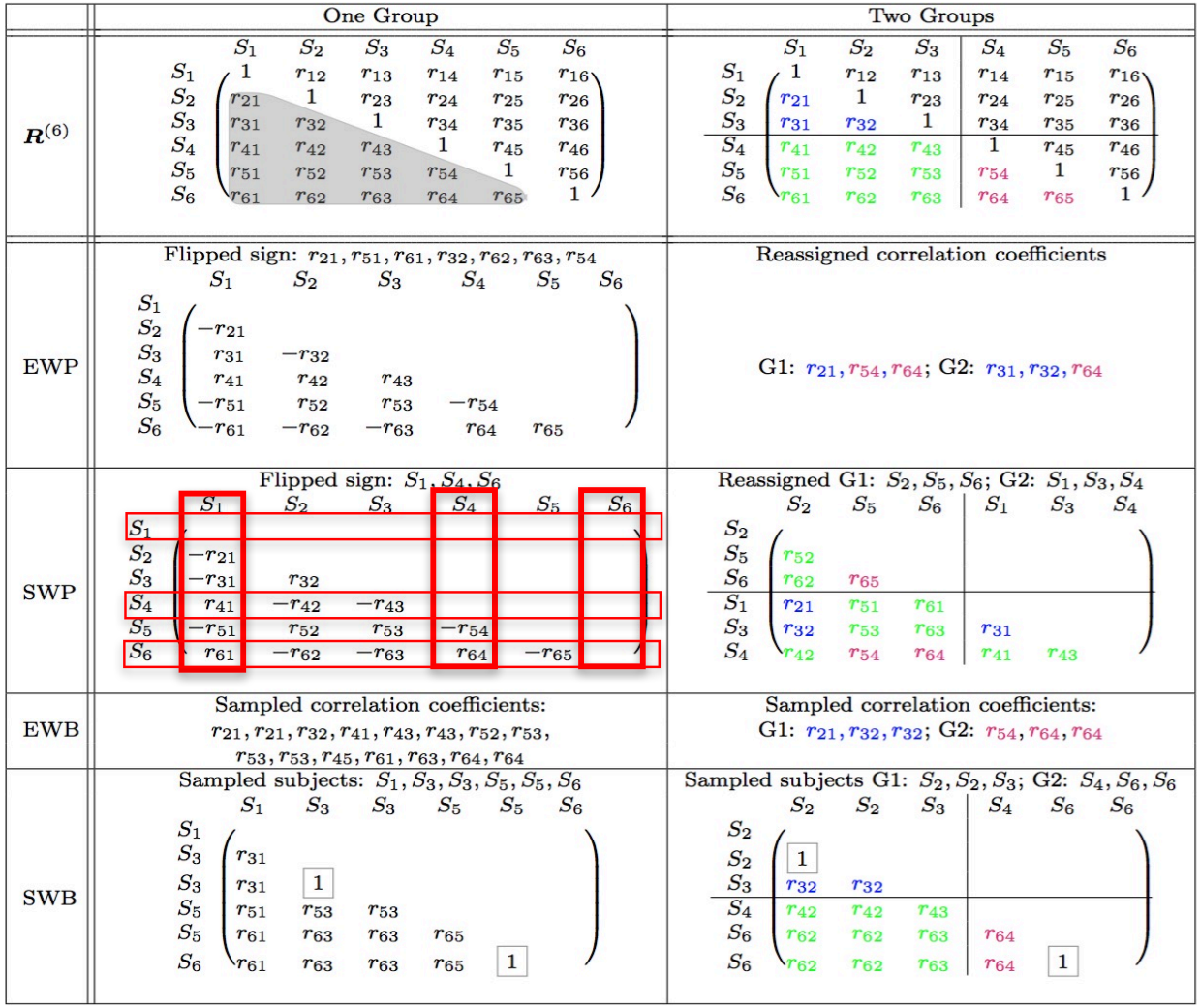
- Various nonparametric methods
  - Permutations: null distribution via randomization across space (voxels) and time (e.g., circularly shifting each subject's time series by a random lag)
    - Matlab package: **ISC Toolbox** (Kauppi et al, 2014)

  - Leave one out (LOO): Kauppi et al, 2010
    - Compute ISC of a subject between a voxel's BOLD time course in the subject and the average of that voxel's time course in the remaining subjects
    - Perform Student *t*-test on the LOO ISC values

- **All these methods have poor FPR controllability**

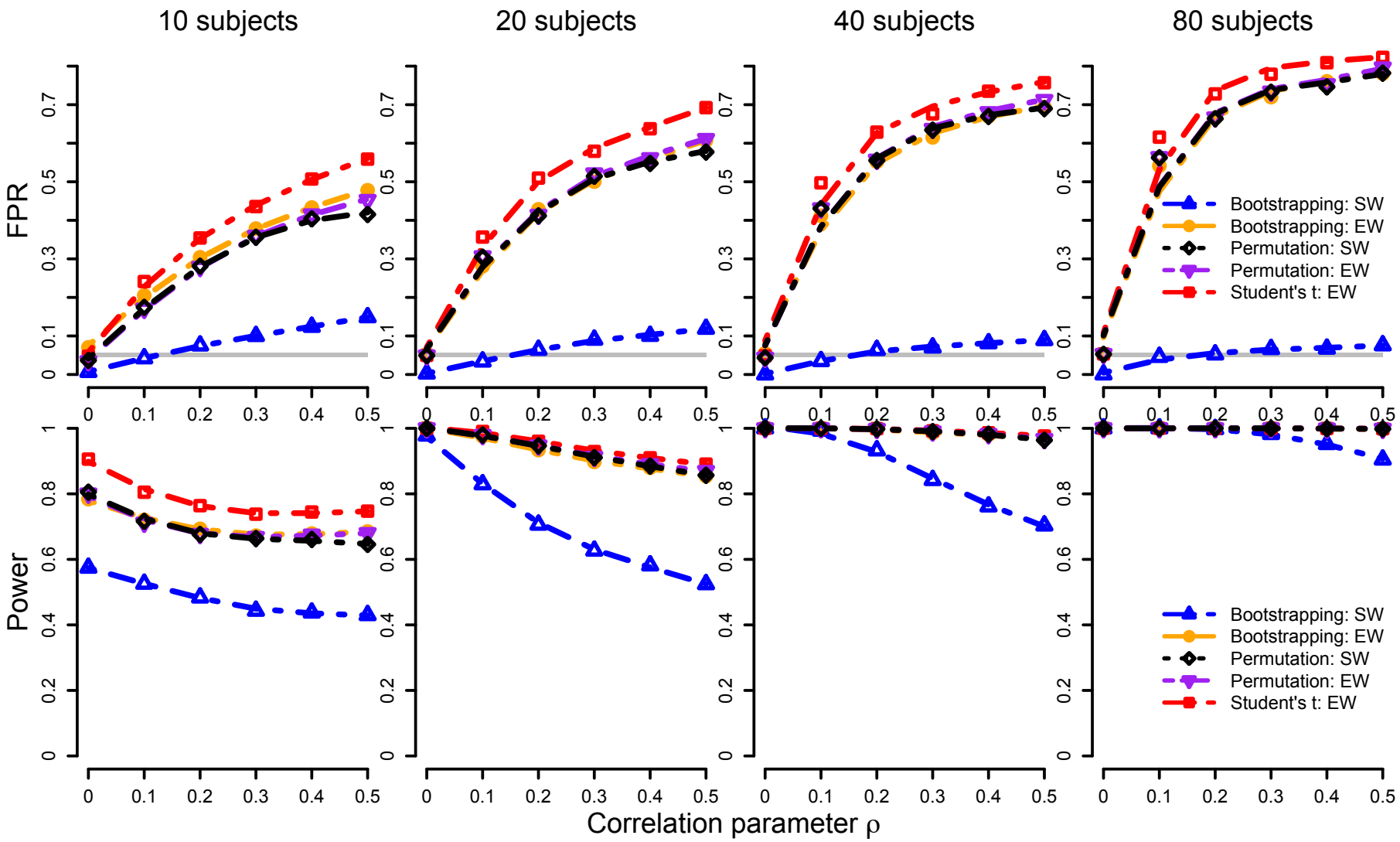
ISC group analysis: exploration with new nonparametric approaches

- Schematic demo of how different methods work



Chen et al, 2016a. Untangling the relatedness among correlations, part I: Nonparametric approaches to inter-subject correlation analysis at the group level. Neuroimage (in press).

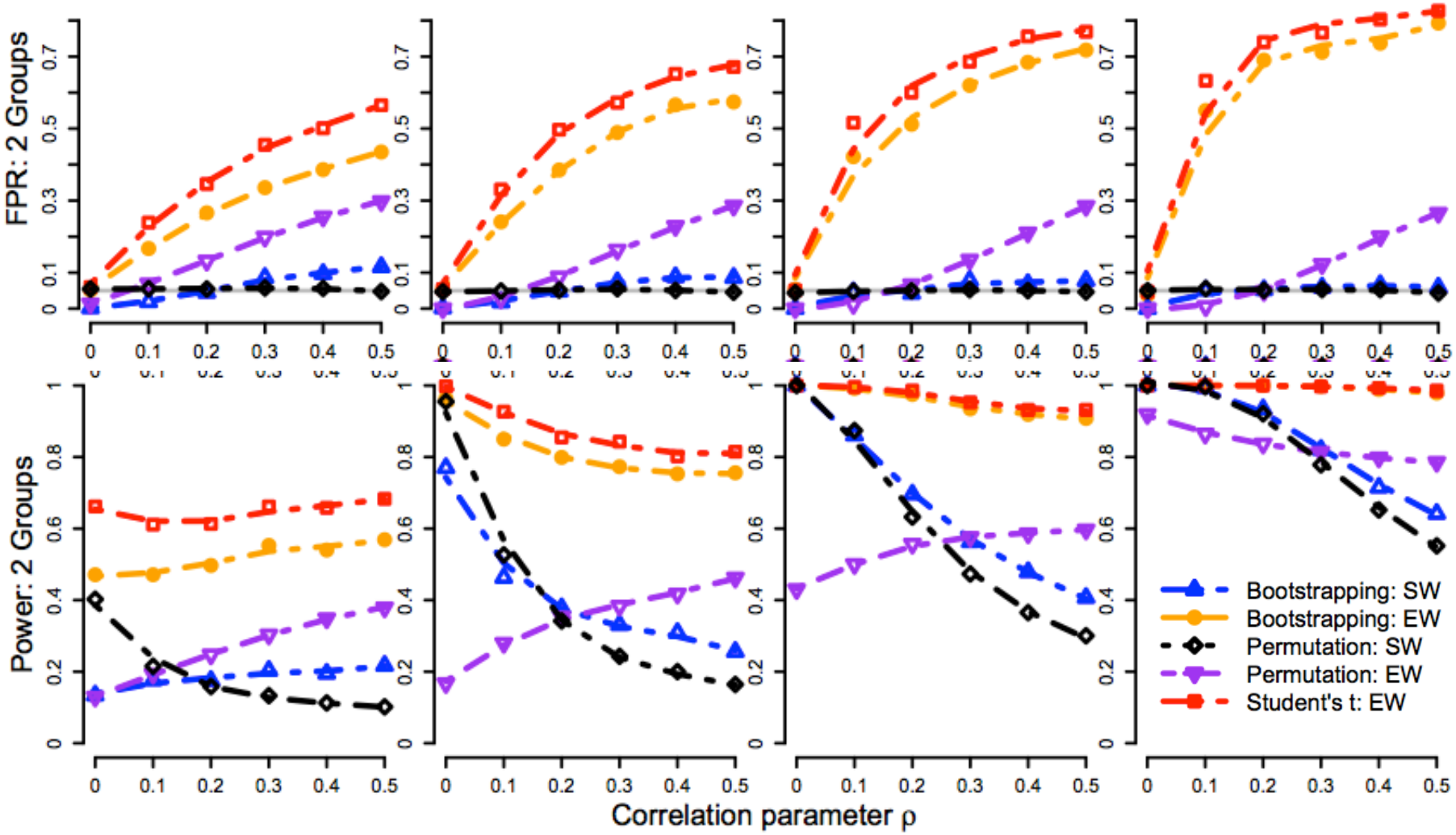
New nonparametric approaches: simulations



Conclusion: SWB acceptable for one group

Chen et al, 2016a. Untangling the relatedness among correlations, part I: Nonparametric approaches to inter-subject correlation analysis at the group level. Neuroimage (in press).

New nonparametric approaches: simulations

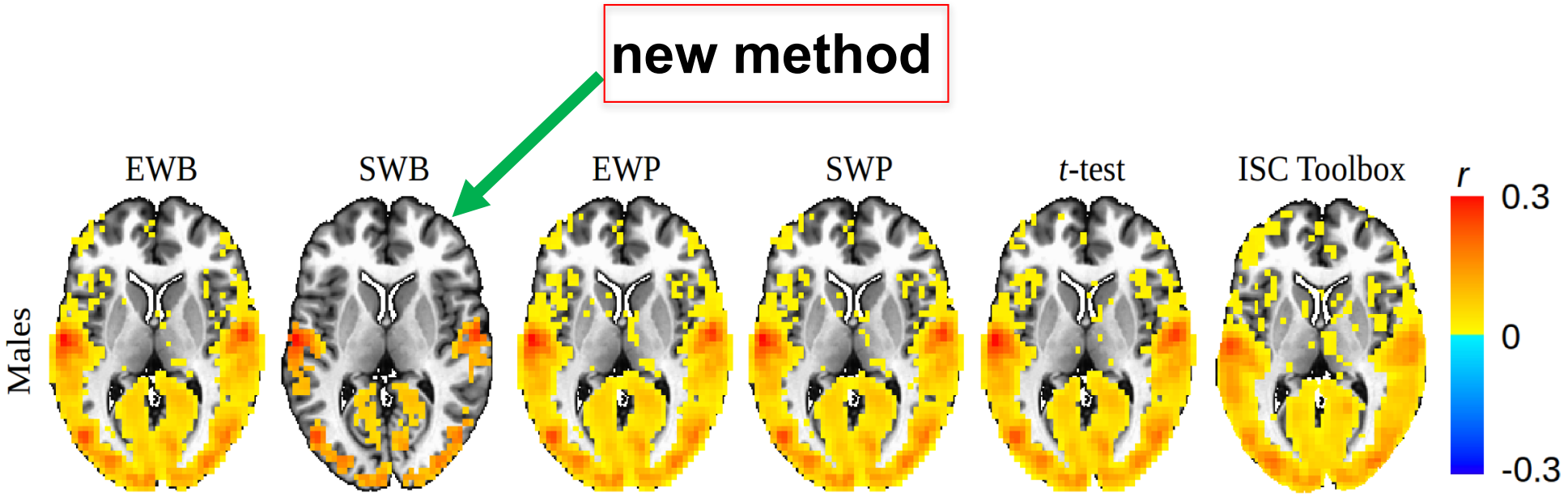


Conclusion: SWP ideal for group comparisons

Chen et al, 2016a. Untangling the relatedness among correlations, part I: Nonparametric approaches to inter-subject correlation analysis at the group level. Neuroimage (in press).

New nonparametric approaches: real data

- One group: 24 male subjects
- 6 movie clips, 406 time points



- Similar results for group comparisons with **SWP**

- Results with real data are consistent with simulation results

Chen et al, 2016a. Untangling the relatedness among correlations, part I: Nonparametric approaches to inter-subject correlation analysis at the group level. Neuroimage (in press).

Linear mixed-effects modeling (LME)

- Modeling via effect partitioning: crossed random-effects LME

$$z_{ij} = b_0 + \theta_i + \theta_j + \epsilon_{ij}, \quad i \neq j$$

$$\theta_i, \theta_j \overset{iid}{\sim} G(0, \zeta^2) \text{ and } \epsilon_{ij} \overset{iid}{\sim} G(0, \eta^2)$$

cross-subject **within-subject**

- Characterizing the relatedness among ISCs via LME

$$\rho = Corr(z_{ij}, z_{jl}) = \frac{Cov(z_{ij}, z_{jl})}{\sqrt{Var(z_{ij})Var(z_{jl})}} = \frac{\zeta^2}{2\zeta^2 + \eta^2}$$

$$0 \leq \rho = \frac{\zeta^2}{2\zeta^2 + \eta^2} = \frac{\zeta^2}{\sigma^2} \leq 0.5$$

Chen et al, 2016b. Untangling the Relatedness among Correlations, Part II: Inter-Subject Correlation Group Analysis through Linear Mixed-Effects Modeling. Neuroimage (in press).

Linear mixed-effects modeling (LME)

- Formulation: crossed random-effects LME

$$z_{ij} = b_0 + \theta_i + \theta_j + \epsilon_{ij}, \quad i \neq j$$

$$\theta_i, \theta_j \overset{iid}{\sim} G(0, \zeta^2) \text{ and } \epsilon_{ij} \overset{iid}{\sim} G(0, \eta^2)$$

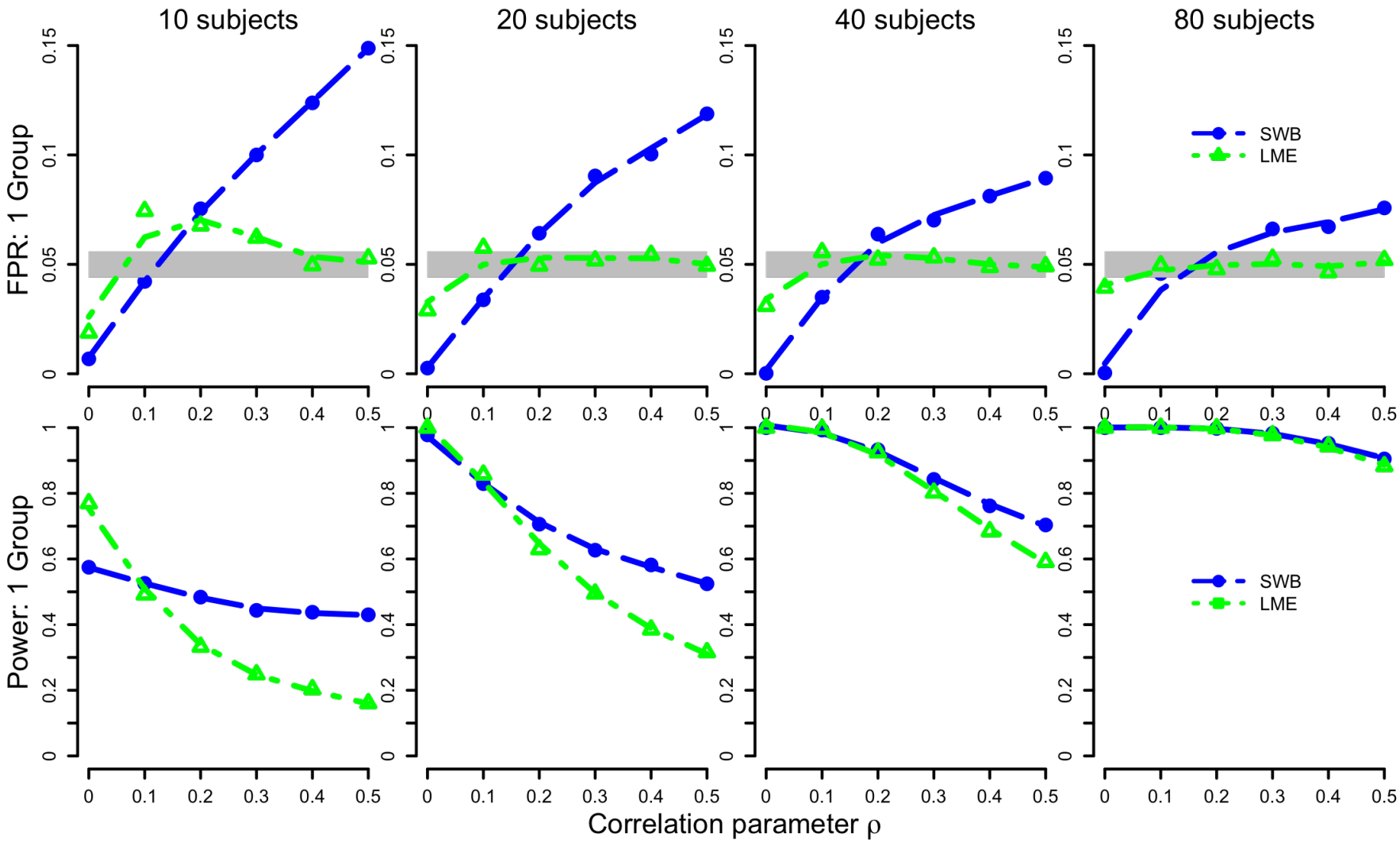
- Extendibility/flexibility of LME
  - Easy to incorporate explanatory variables: between- and within-subject factors (or quantitative covariates) similar to extension of *t*-test to GLM

- Data characterization and model quality: unavailable for nonparametric approaches
  - Cross-subject variance ζ^2
  - Within-subject variance η^2
  - Relatedness of ISCs ρ

$$0 \leq \rho = \frac{\zeta^2}{2\zeta^2 + \eta^2} = \frac{\zeta^2}{\sigma^2} \leq 0.5$$

Chen et al, 2016b. Untangling the Relatedness among Correlations, Part II: Inter-Subject Correlation Group Analysis through Linear Mixed-Effects Modeling. Neuroimage (in press).

LME: simulations

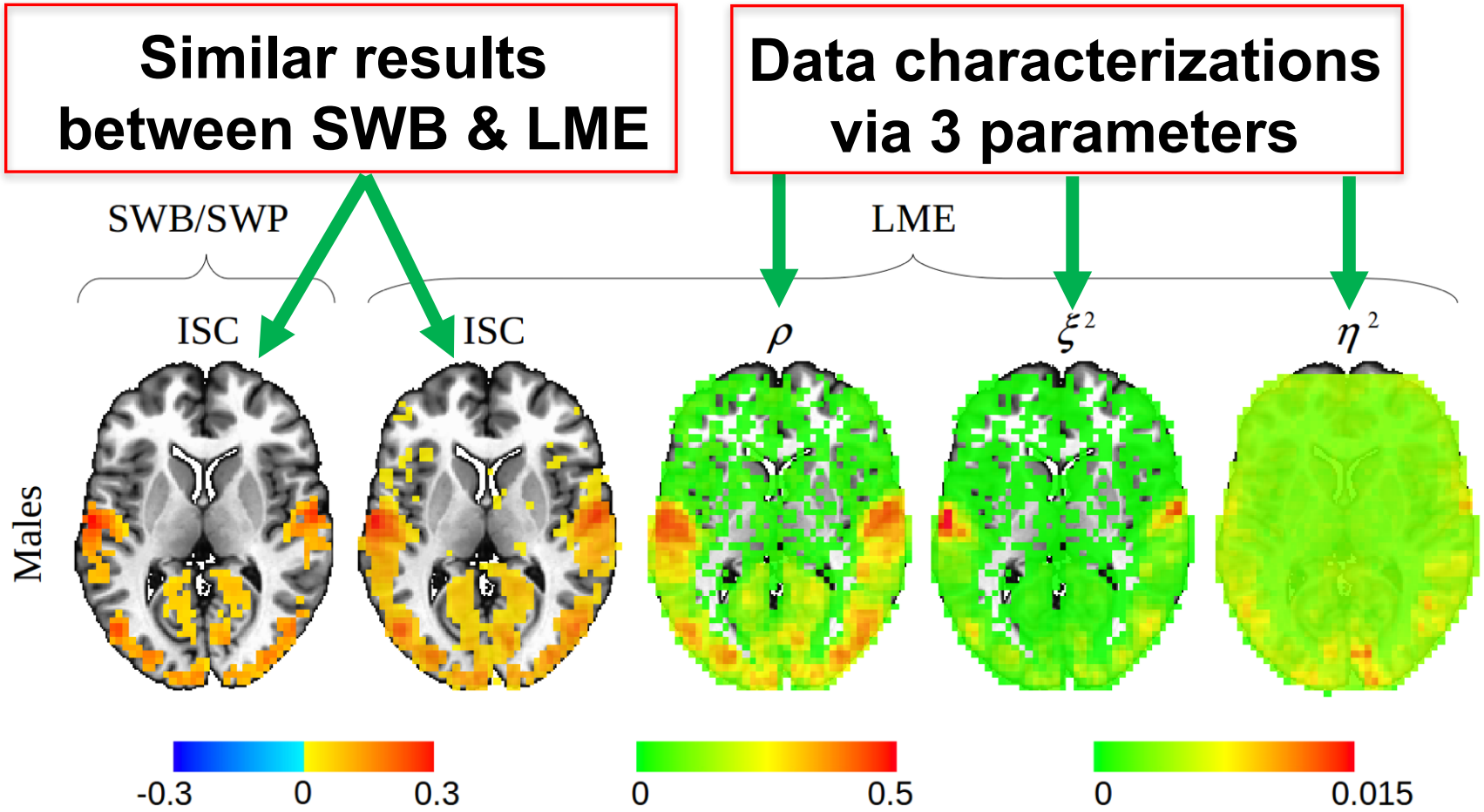


LME: better FPR controllability than SWB for one group, and similar to SWP for group comparisons

Chen et al, 2016b. Untangling the Relatedness among Correlations, Part II: Inter-Subject Correlation Group Analysis through Linear Mixed-Effects Modeling. Neuroimage (in press).

LME: real experiment data

- 48 subjects (24 males, 24 females)
- 6 movie clips, 406 time points



Chen et al, 2016b. Untangling the Relatedness among Correlations, Part II: Inter-Subject Correlation Group Analysis through Linear Mixed-Effects Modeling. Neuroimage (in press).

Benefits of naturalistic paradigm

- Similar to resting-state FMRI

- Extendable to other modalities
  - EEG, MEG, ECoG, fNIRS…

- No presumption about HDR function

- More controlled and engaging (especially for children)

- Practical benefit: subject less likely to fall asleep

- Analysis benefits
  - Less vulnerable to head motion effects
  - Statistically more powerful
  - Not dependent on seed selection (in seed-based approach)
  - Not dependent on dimension reduction and component selection
  - Well-fit by powerful LME with crossed random effects paradigm

Overview

- Basic concepts
  - Why do we need to do group analysis?
  - Factor, quantitative covariates, main effect, interaction, …
- Various group analysis approaches
  - Regression (*t*-test): 3dttest++, 3dMEMA, 3dttest, 3RegAna
  - AN(C)OVA: 3dANOVAx, 3dMVM, GroupAna
  - Quantitative covariates: 3dttest++, 3dMEMA, 3dMVM, 3dLME
  - Impact & consequence of SFM, SAM, and SEM
- Miscellaneous
  - Issues regarding result reporting
  - Intra-Class Correlation (ICC)
  - Inter-Subject Correlation (ISC)
  - Nonparametric approach and fixed-effects analysis
- No routine statistical questions, only questionable routines!