Group Analysis

File: **GroupAna.pdf**

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SSCC/NIMH/NIH/HHS

FMRI Study Pipeline

Experiment Design

↓

Scanning

↓

Pre-Processing

↓

Individual Subject Analysis

↓

Group Analysis

↓

Post-Processing: clusterization,
ROI analysis, connectivity, …

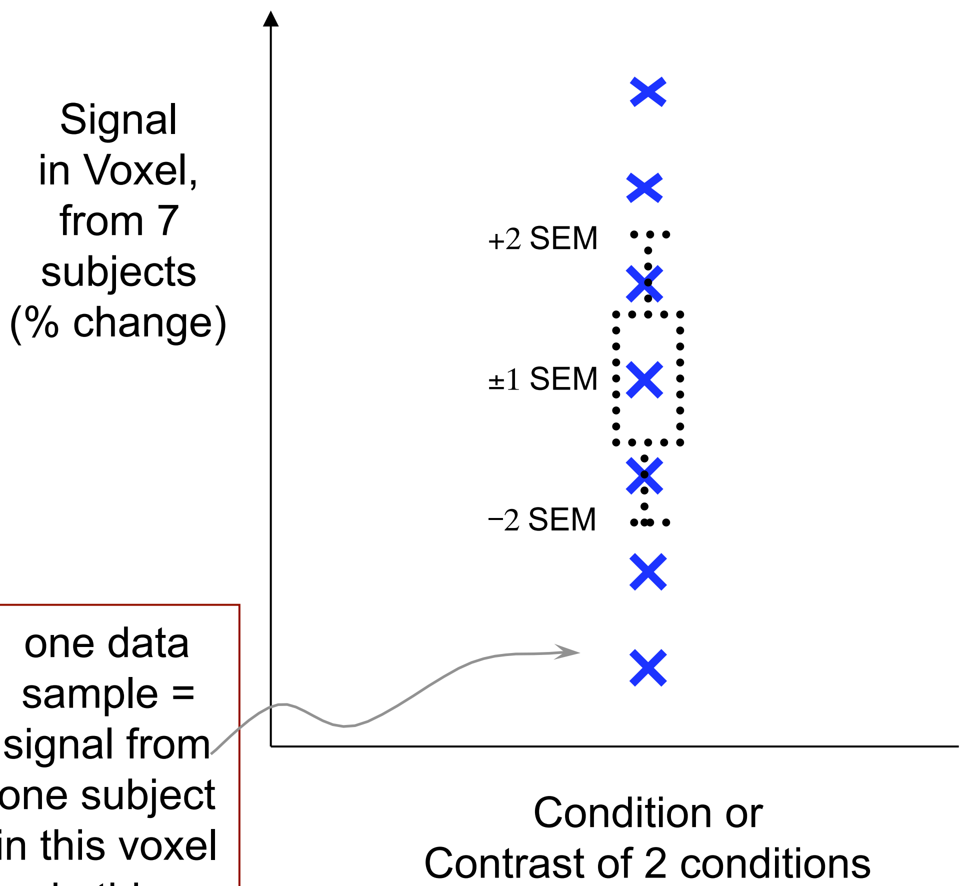
Preview

- Introduction: basic concepts
  - 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 and fixed-effects analysis
  - Inter-Subject Correlation (ISC) analysis

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



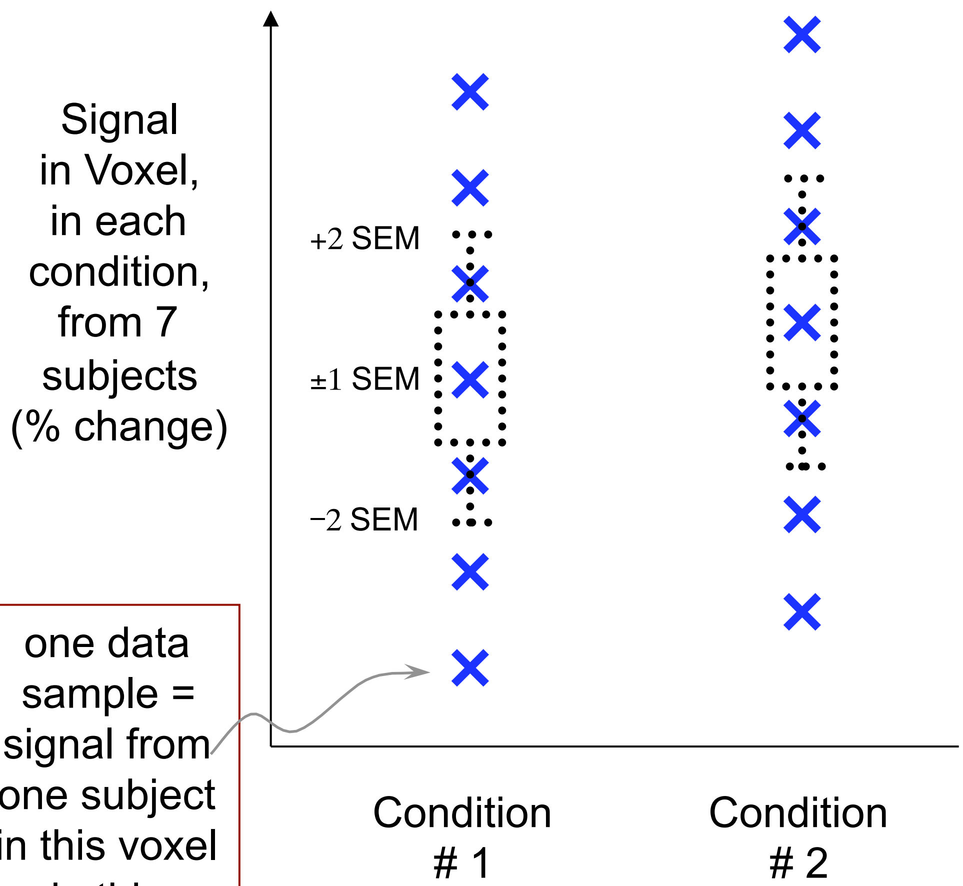
one data sample = signal from one subject in this voxel in this condition

- <u>SEM</u> = 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

- <u>statistically</u> <u>significantly</u> different from 0!

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

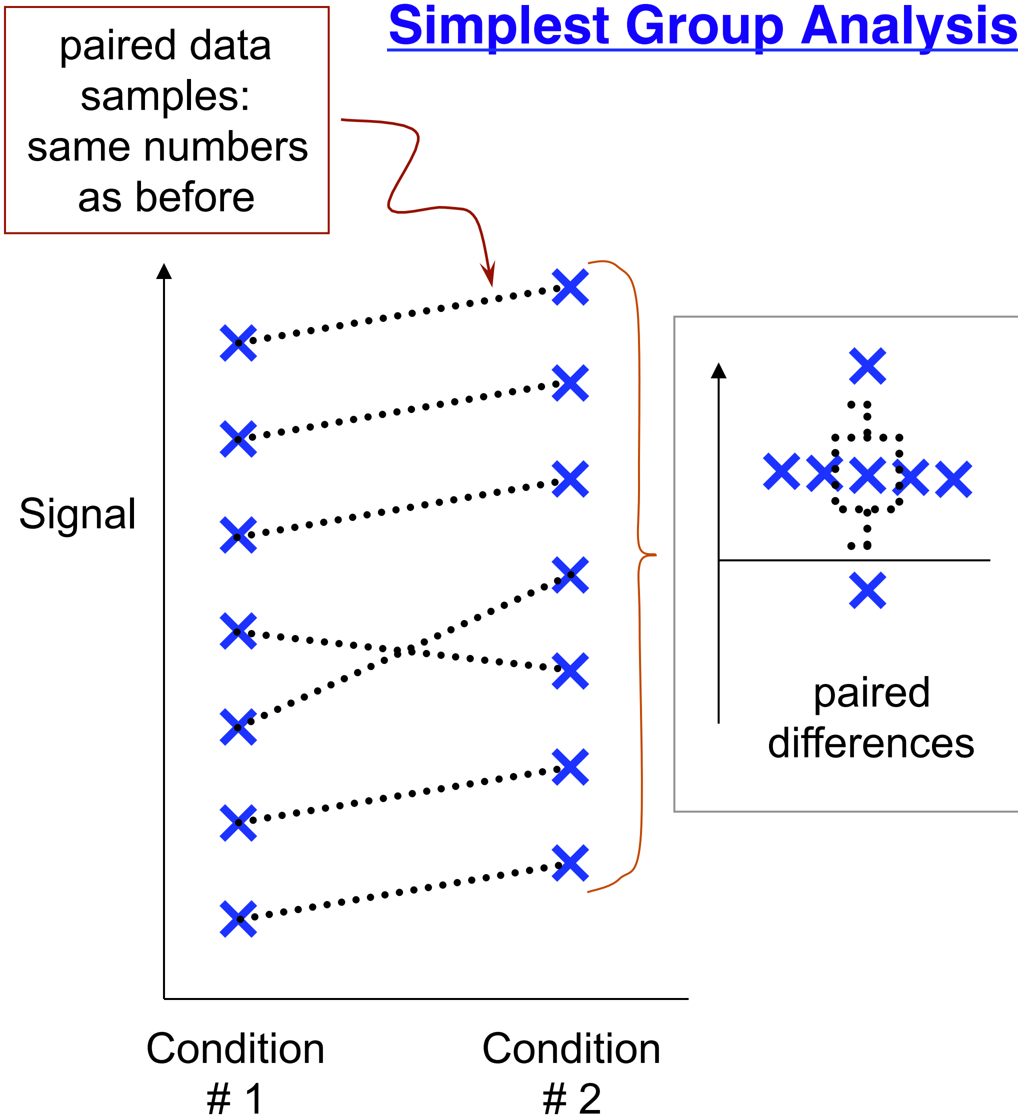


one data sample = signal from one subject in this voxel in this condition

- Condition = some way to categorize data (*e.g.*, stimulus type, drug treatment, day of scanning, subject type, …)
- 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



- 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
  - What we have: (β_1, β_2, …, β_{10})=(1.13, 0.87, …, 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 0.00 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

- Conventional: voxel-wise (brain) or node-wise (surface)
  - Proper model to account for cross-and within-subject variability
- 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
- Statistically insignificant but the effect magnitude is suggestive
  - Sample size
  - Alignment of different subjects' brain images

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
  - When quantitative covariates are involved
- Advantages of big models: AN(C)OVA, GLM, LME
  - All tests in one analysis (vs. piecemeal *t*-tests)
  - Omnibus *F*-statistics
  - Power gain: combining subjects across groups for estimates of signal *and* noise parameters (*i.e.*, variances and correlations)

Terminology: Explanatory variables

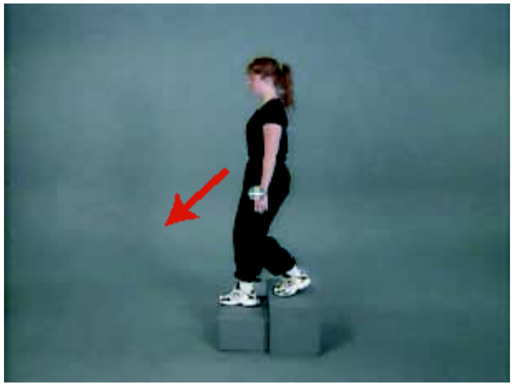
- **Response/Outcome variable** (HDR): regression β coefficients
- **Factor**: categorical, qualitative, nominal or discrete variable
  - Categorization of conditions/tasks
    - Within-subject (repeated-measures) factor
  - Subject-grouping: Group of subjects (sex, normal/patients)
    - Between-subjects factor
    - Gender, patients/controls, genotypes, …
  - 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, *etc*.

Terminology: Fixed effects

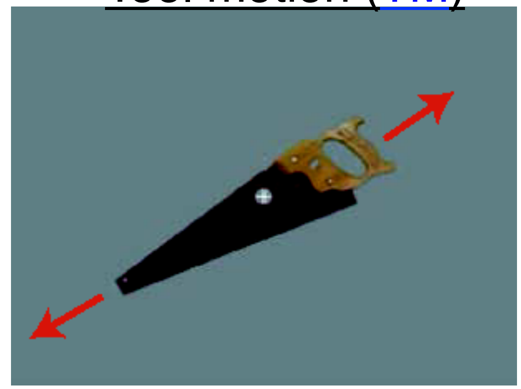
- Fixed-effects factor: categorical (qualitative or discrete) variable
  - Treated as a fixed variable (constant to be estimated) in the model
    - Categorization of conditions/tasks (modality: visual/auditory)
      - Within-subject (repeated-measures) factor: 3 emotions
    - Subject-grouping: Group of subjects (gender, normal/patients)
      - Between-subject factor
  - All levels of a factor are of interest
    - main effect, contrasts among levels
  - Fixed in the sense of statistical inferences
    - Apply only to the specific levels of the factor
      - 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

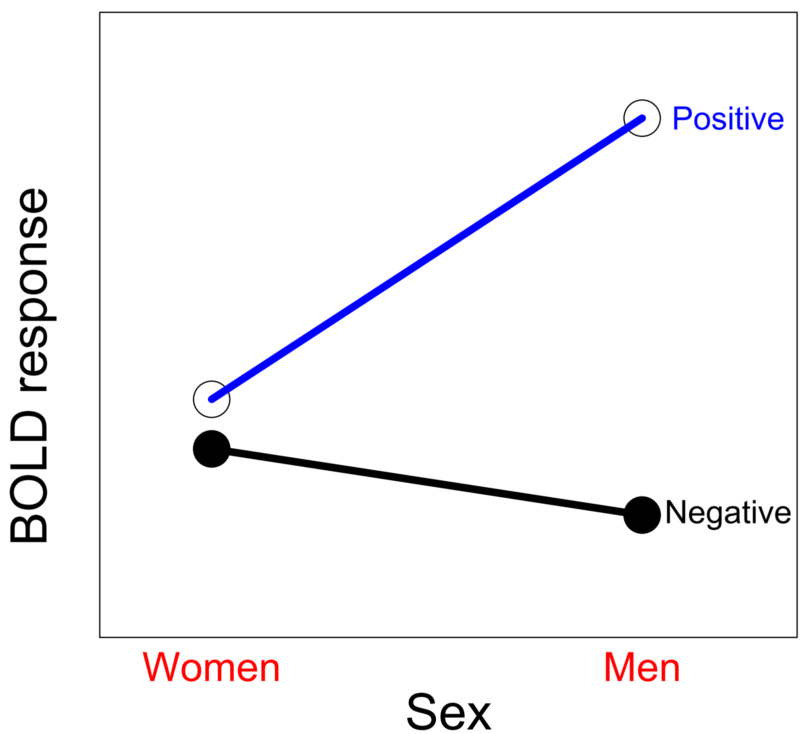
- Random factor/effect
  - Random variable in the model: exclusively used for subject in FMRI
    - average + effects attributable to each subject: *e.g.* $N(\mu, \tau^2)$
    - Requires enough subjects to estimate properly
  - Each individual subject effect is of NO interest
    - Group response = 0.92%, subject 1 = 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 model: decomposing each subject's response
  - Fixed (population) effects: universal constants (immutable): $\boldsymbol{\beta}$

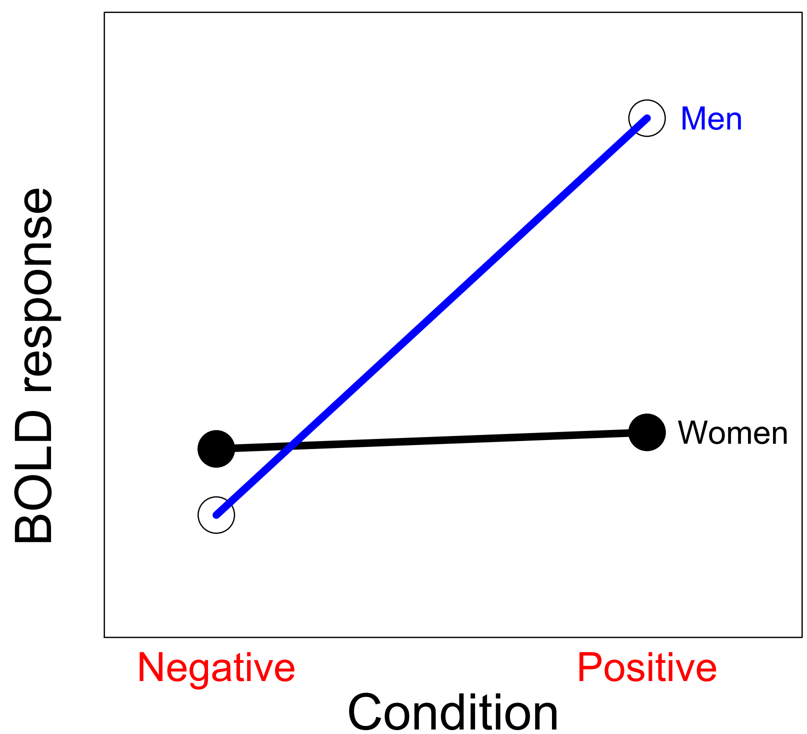
$$\boldsymbol{y}_i = X_i\boldsymbol{\beta} + Z_i\boldsymbol{b}_i + \boldsymbol{\epsilon}_i$$

  - Random effects: individual subject's deviation from the population (personality: durable for that 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



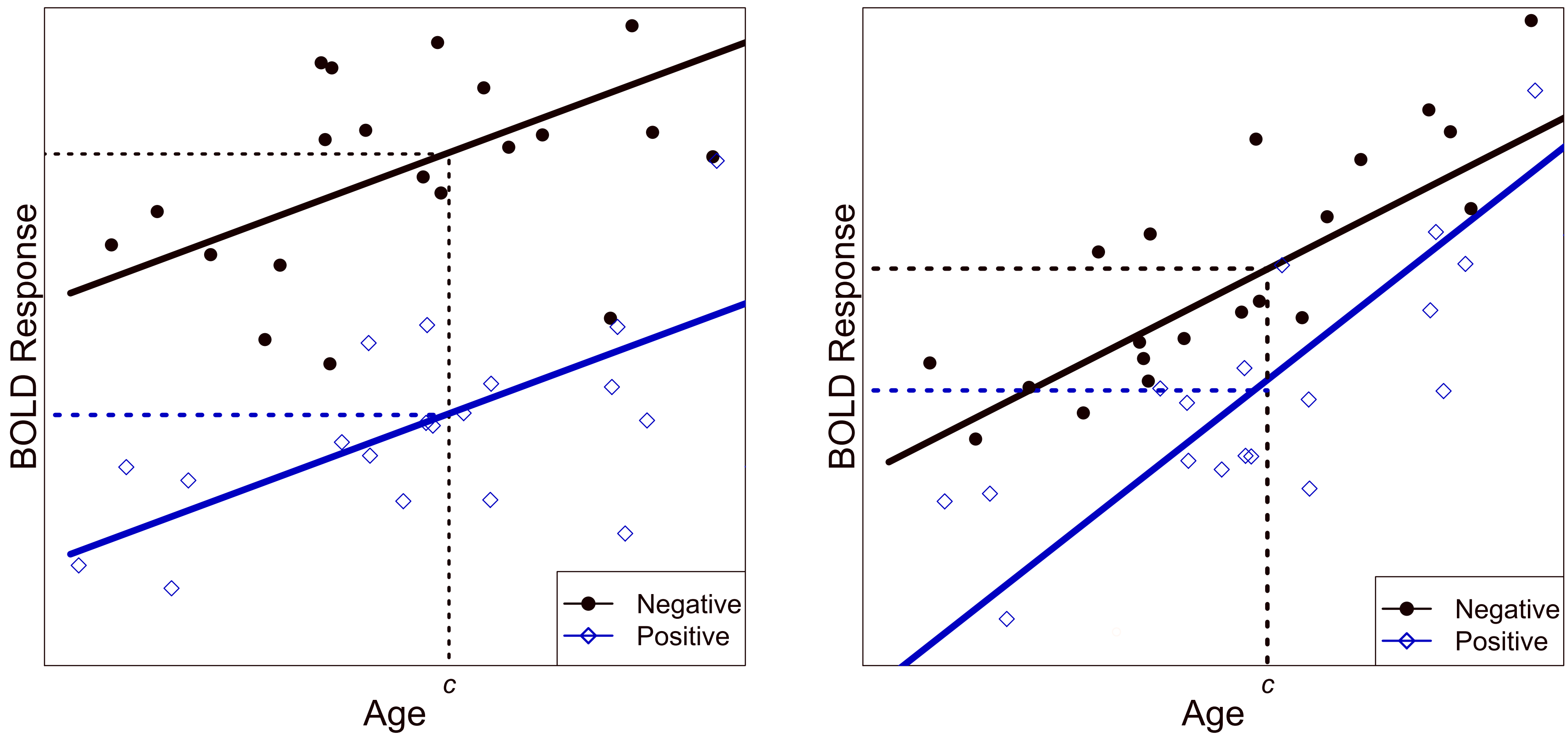


Terminology: Interaction

- Interactions: ≥ 2 factors
  - May become very difficult 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 ***β***s) 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
- Alternative: taking both effect estimates and *t*-statistics
  - *t*-statistic contains precision information about effect estimates
  - Each subject's ***β*** is weighted based on precision of effect estimate (more precise ***β***s get more weight)
- All models in common use are some type of linear model
  - *t*-test, AN(C)OVA, LME, MEMA
  - Partition each subject's effect into multiple components

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

- ✧ Explanatory variables
  - 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

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

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
- ✧ **Problematic**: 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

Our 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

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

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

Why use β 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 (might vary per subject)
    - 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 25 times larger than the former?
  - Distributional considerations – not very Gaussian (normal)
- ✧ 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 (we hope) 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
      - That is, do 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
  - Multiple regression: one group + one 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
  - One group against a constant: 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 basis functions: **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 \
 -setA 'FP+tlrc[Vrel#0_Coef]'              \
       'FR+tlrc[Vrel#0_Coef]'              \
       ......
       'GM+tlrc[Vrel#0_Coef]'
```

- **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
```

Dataset value = 0 ➔ treat it as missing

Two-Sample Case

- Two groups of subjects ($n \geq 10$ each); for example: 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**, **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 $\boldsymbol{\beta}$s
- 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                 \
  -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** (also somewhat hard work)

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** (two-way ANOVA with one 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**

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** | **Data layout**

```
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
```

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 $\boldsymbol{\beta}$ values to group analysis (MVM)
- Adjusted-Shape method (**ASM**) via SPMG2/3
  - Only take the major component $\boldsymbol{\beta}$ to group level
  - ***or***, Reconstruct HRF, and take the effect estimates (*e.g.*, AUC)

Group analysis with multiple basis functions

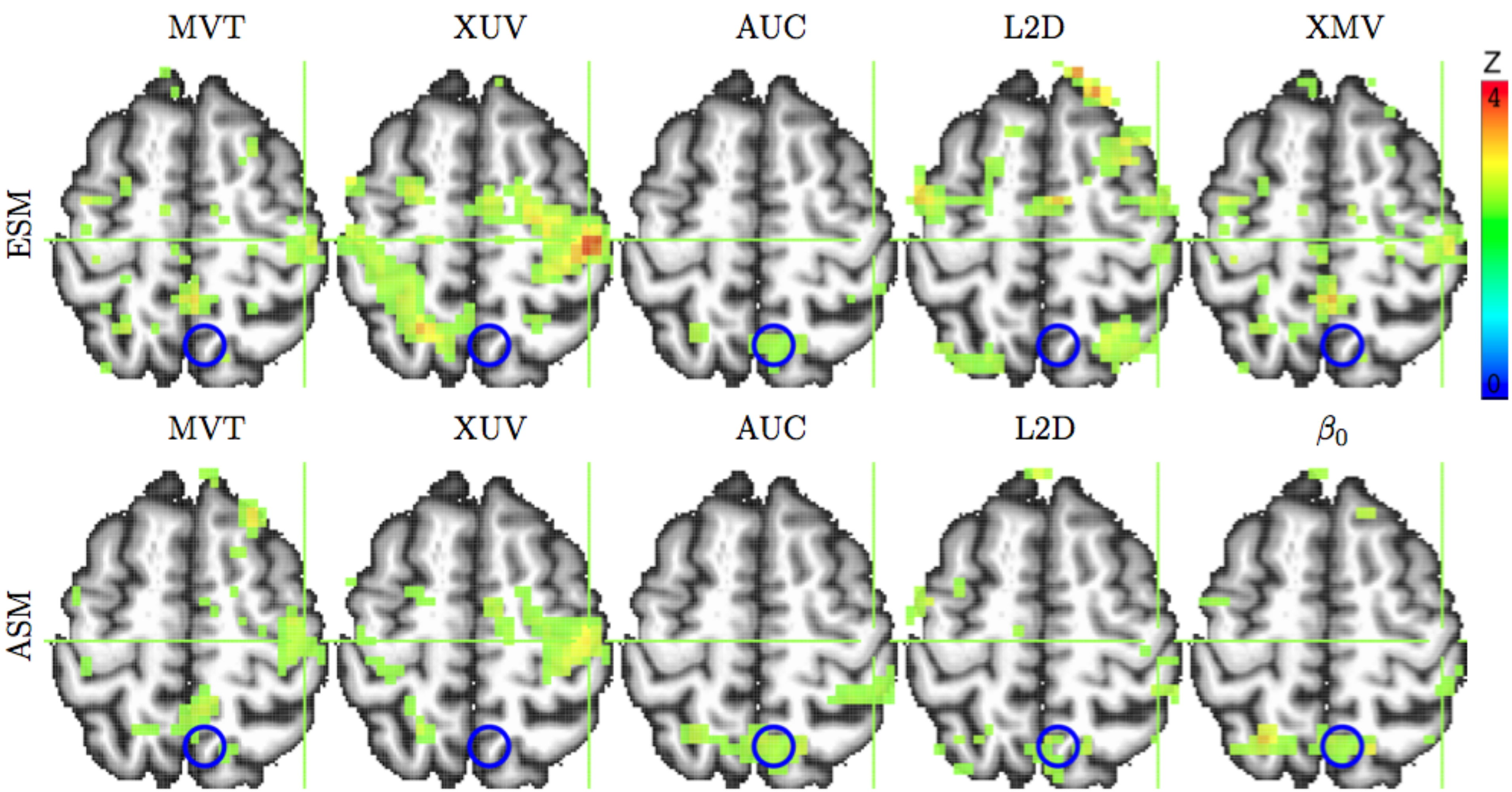
- Analysis with effect estimates at consecutive time grids (from TENT or CSPLIN or reconstructed HRF)
  - Used to be considered very hard to set up (in GLM)
  - Extra variable in analysis: Time = $t_0, t_1, \ldots, t_k$
  - One group of subjects under one condition
    - Accurate null hypothesis is H_0: $\beta_1=0, \beta_2=0, \ldots, \beta_k=0$ (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: $\beta_1^{(1)} - \beta_1^{(2)} = 0, \beta_2^{(1)} - \beta_2^{(2)} = 0, \ldots, \beta_k^{(1)} - \beta_k^{(2)} = 0$
    - 2 conditions: **3dLME**
  - Approximate hypothesis: $\beta_1^{(1)} = \beta_1^{(2)}, \beta_2^{(1)} = \beta_2^{(2)}, \ldots, \beta_k^{(1)} = \beta_k^{(2)}$
    - 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**

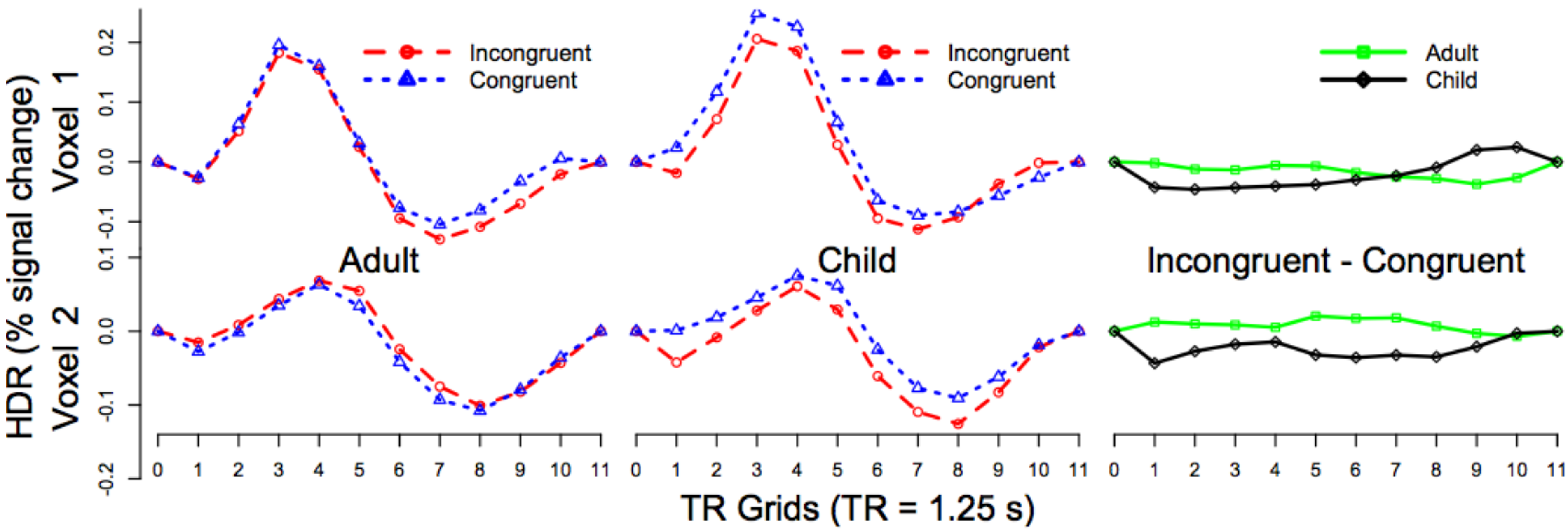
Group analysis with multiple basis functions

- 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 ($\boldsymbol{\beta}_i$) 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)

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

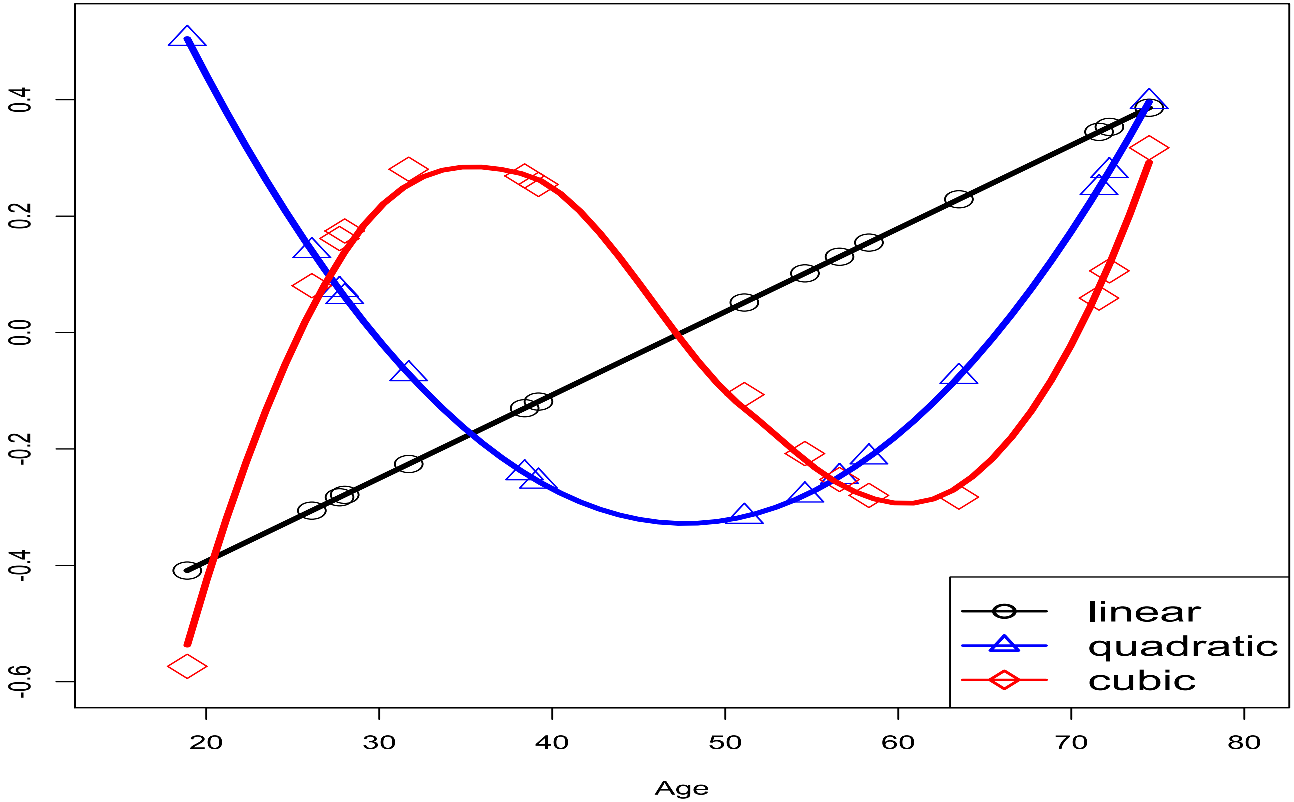
    - Programs: **3dttest++**, **3dMEMA**, **3dMVM**, **3dRegAna**

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



Trend analysis: summary

- **Cross-trials** trend: AM2 single subject analysis with weights
- Modeling with within-subject trend (group level)
  - Run GLT with appropriate weights at individual levels
- 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

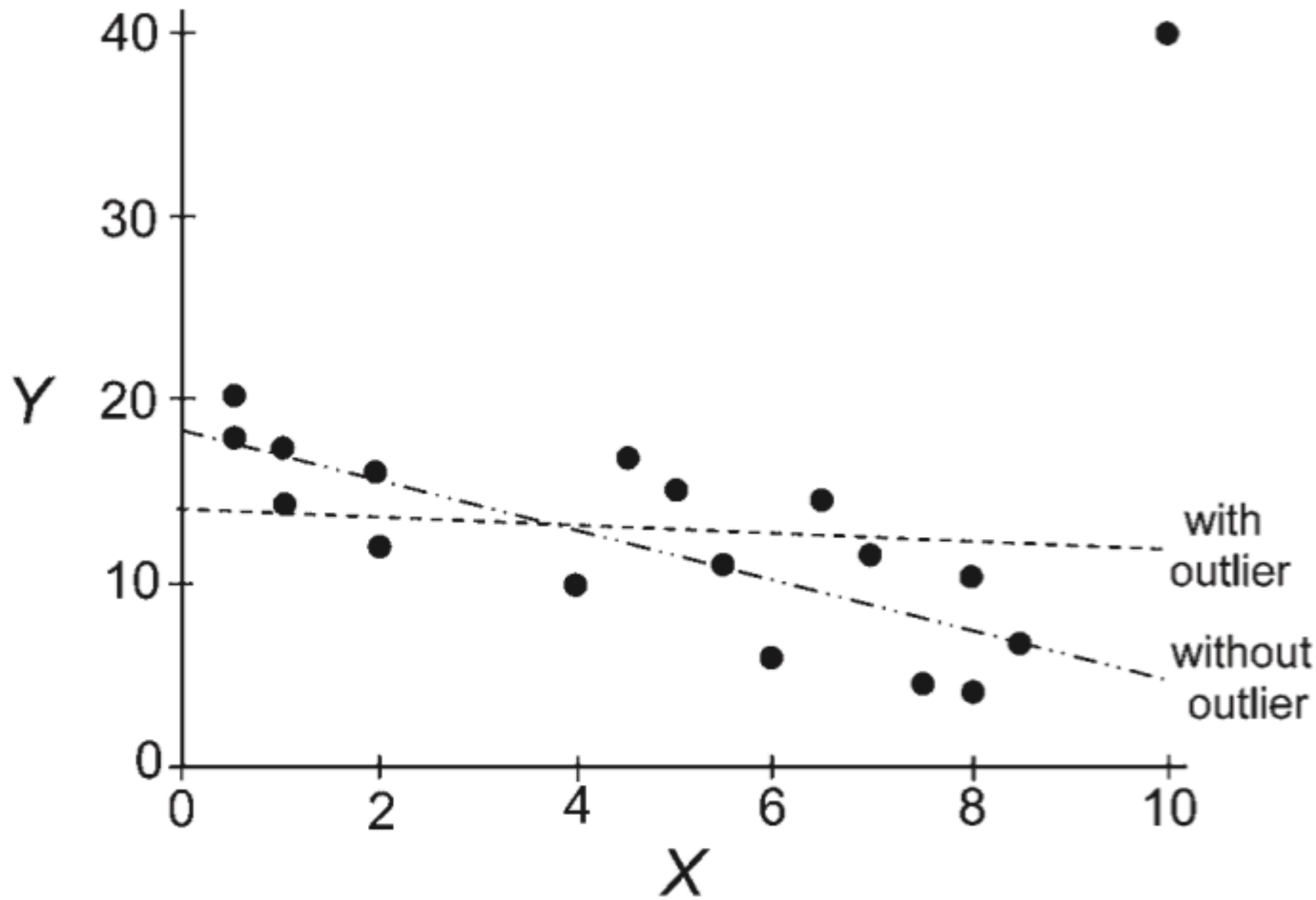
- Covariate: 3 usages
  - Quantitative (vs. categorical) variable of interest
    - Age, IQ, behavioral measures, …
  - Of no interest to the investigator (trying to remove variance)
    - Age, IQ, sex, handedness, scanner,…
  - Any explanatory variables in a model
- Variable selection
  - Infinite candidates 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
  - "Controlling x at …", "holding x constant": centering
  - Regressing out dependence on x?

Caveats with covariate modeling

- Linear regression with few data points: Sensitive to outliers

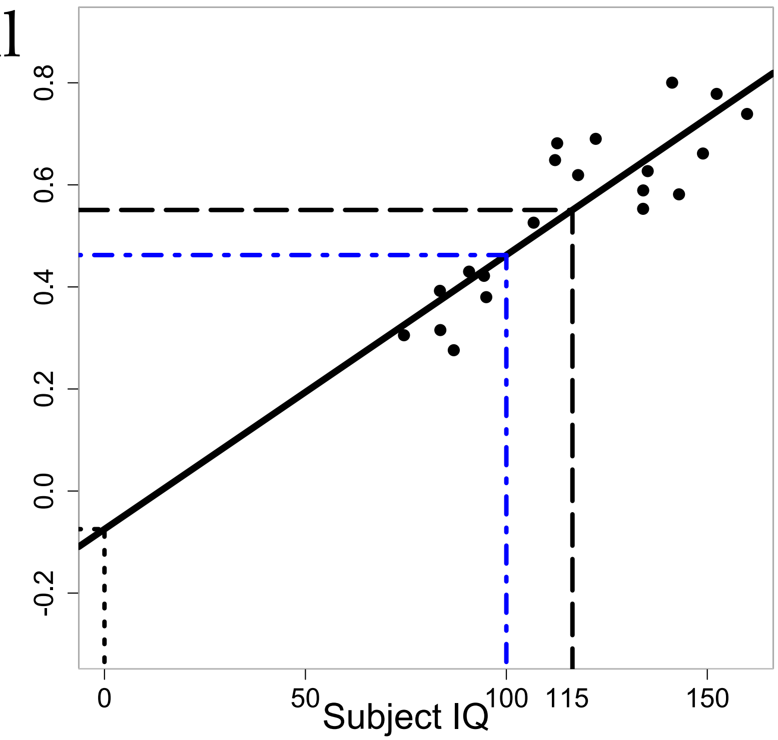


Caveats with covariate modeling

- **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?

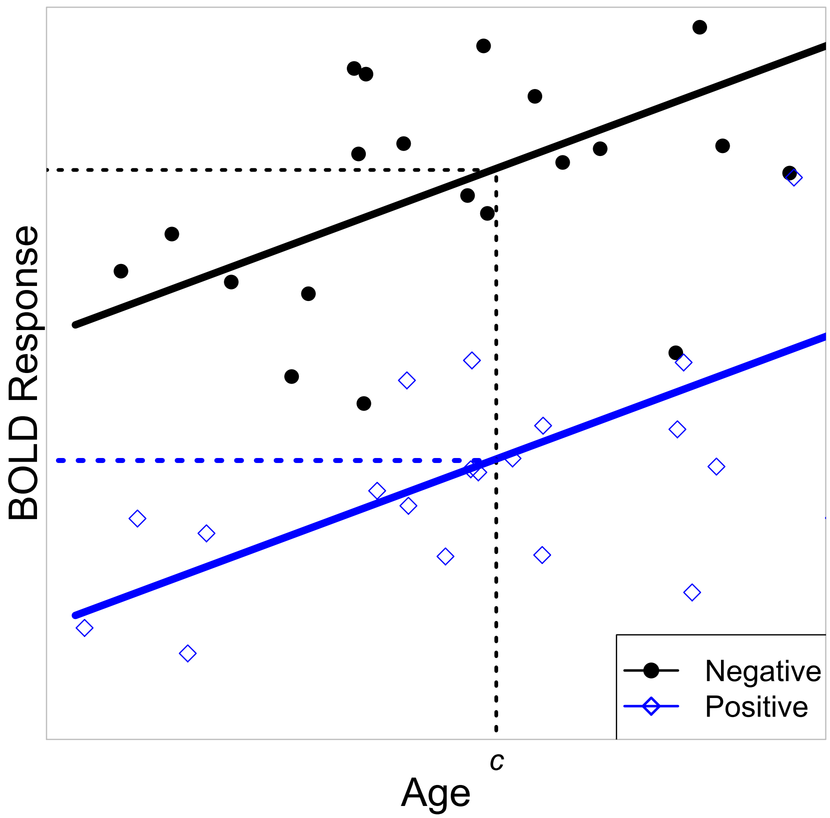


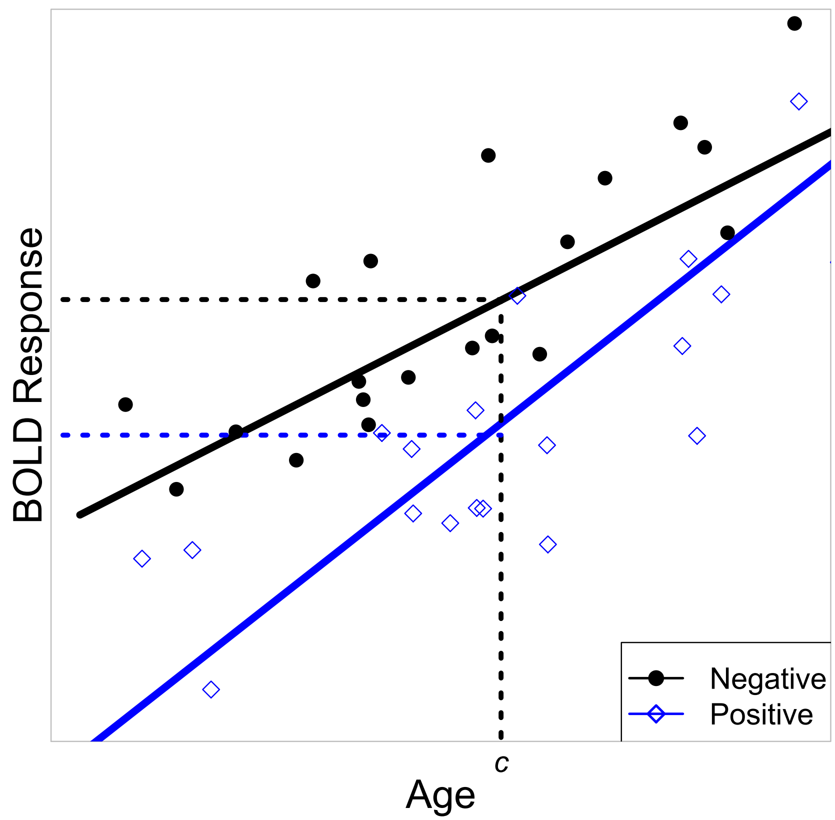
Quantitative variables: subtleties + confusion

- 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?

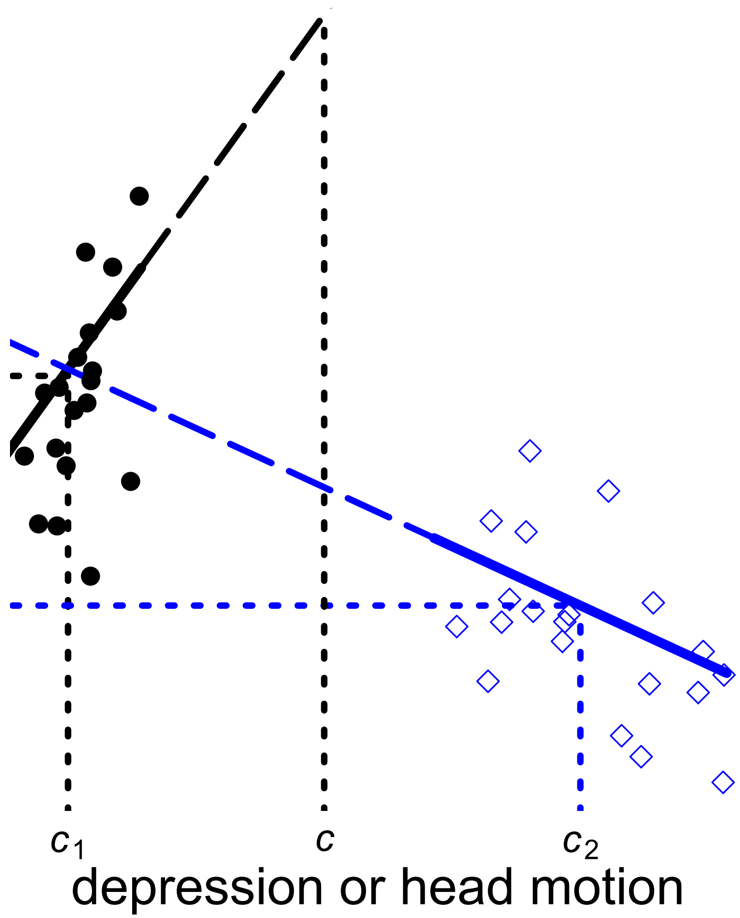




Quantitative variables: subtleties

- Trickiest scenario with two or more groups

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

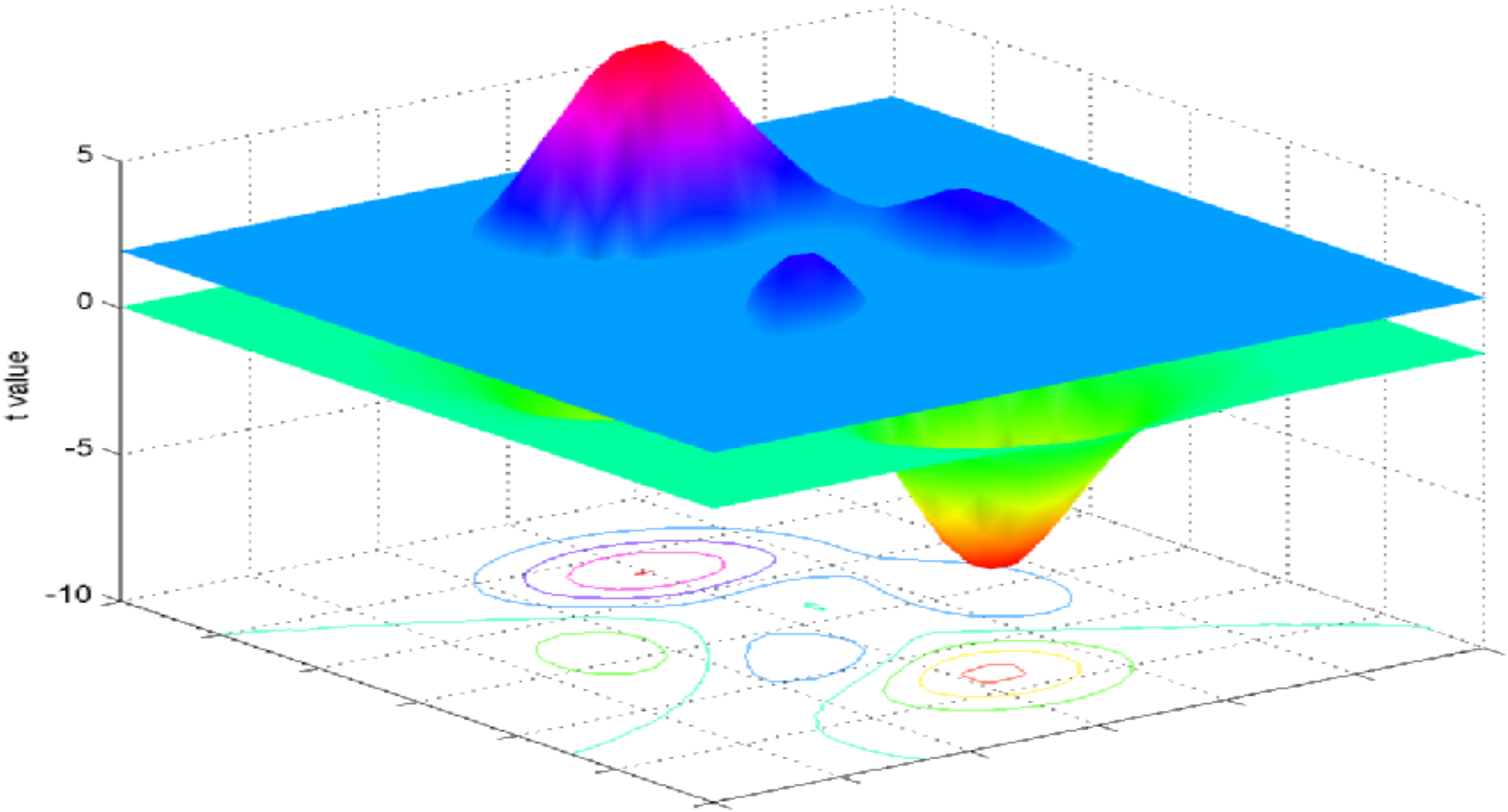


- More at **http://afni.nimh.nih.gov/sscc/gangc/centering.html**

Why should we report response magnitudes?

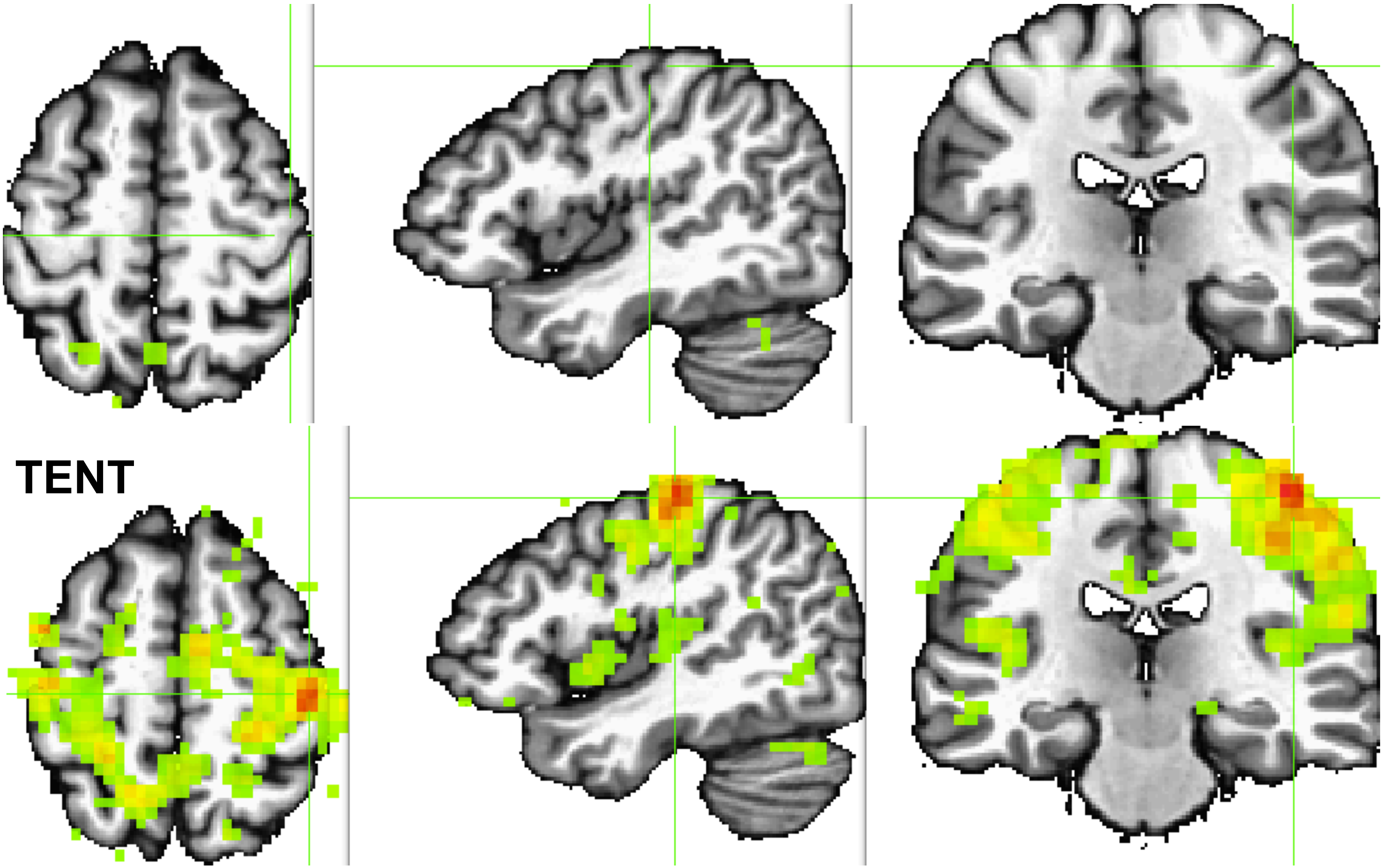
- **Unacceptable** in some fields to report only significance (peak t and smallest p)
- Neuroimaging is an exception currently!
- Obsession in FMRI about p-value!
  - Colored blobs of t-values
  - Peak voxel selected based on peak t-value
- Science is about reproducibility
  - Response amplitude should 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

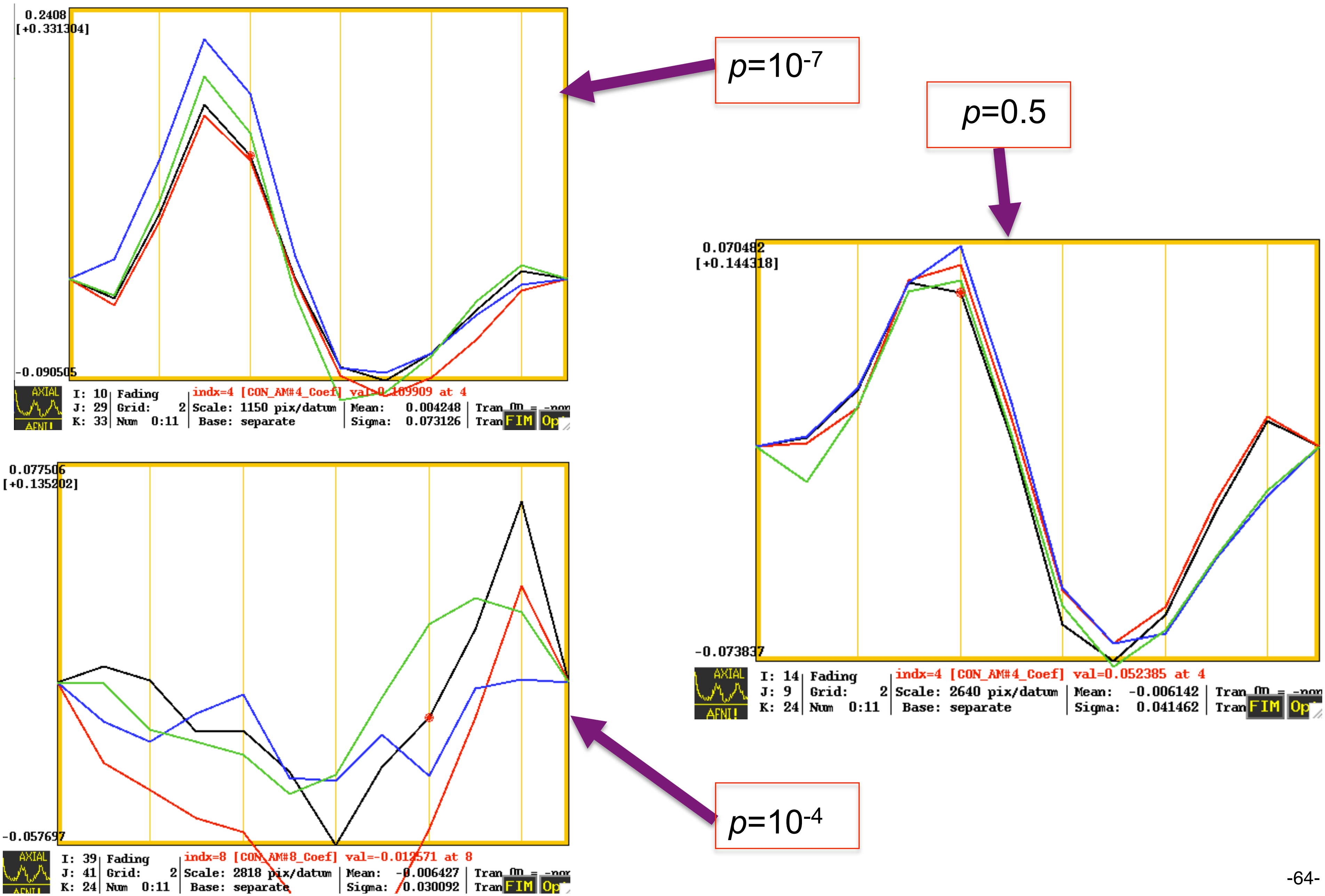


Modeling strategy & results: an example

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



Is *p*-value everything? An example



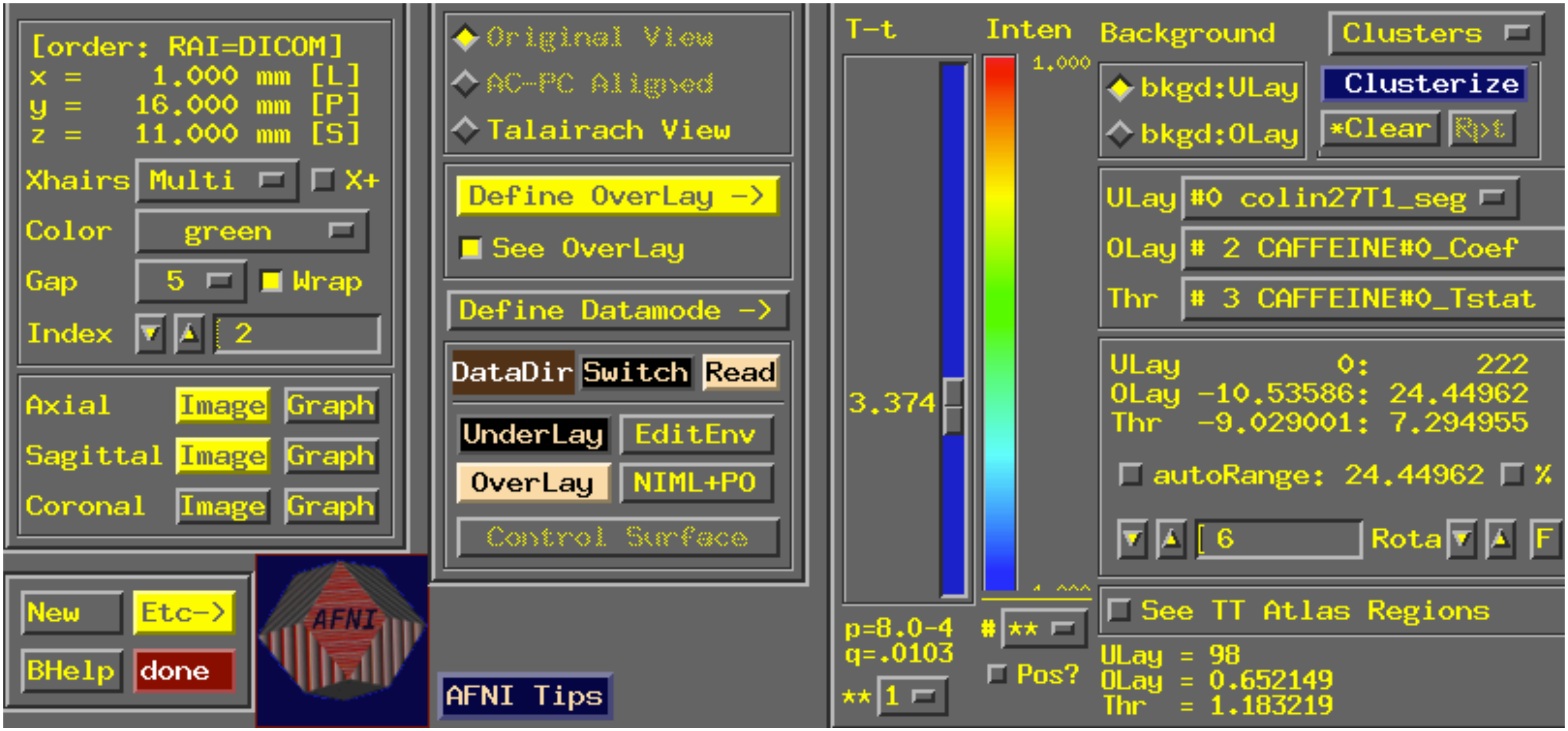
Advantages of ESM

- 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?

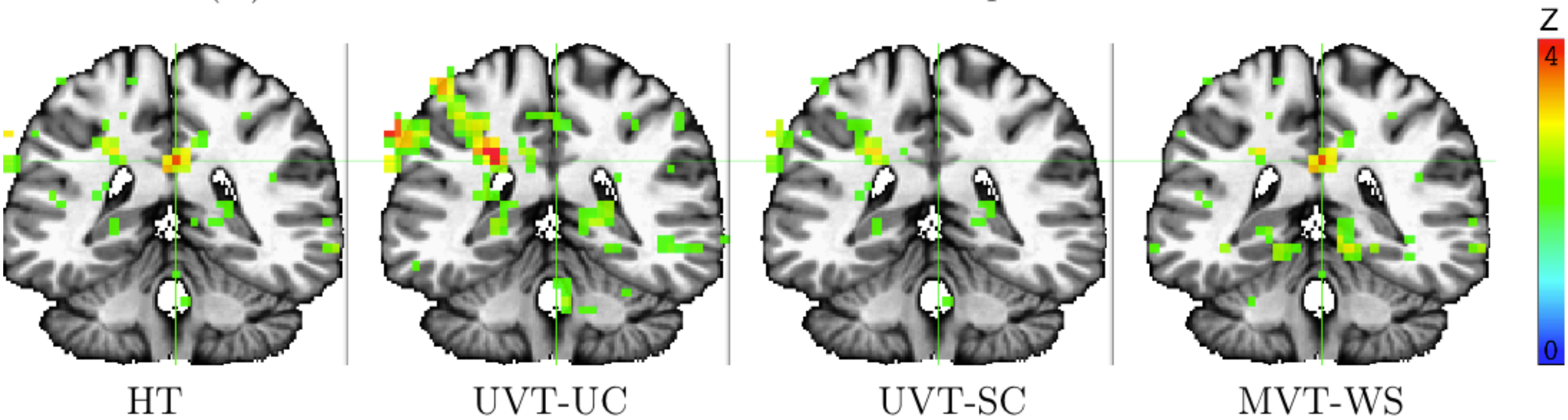
- Two types of correction
  - Multiple testing correction n(MTC): **same** test across brain
    - FWE, FDR, SVC(?)
    - People (esp. reviewers) worship this!
  - Multiple comparisons correction (MCC): **different** tests
    - Happy vs. Sad, Happy vs. Neutral, Sad vs. Neutral
    - Two one-sided *t*-tests: *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

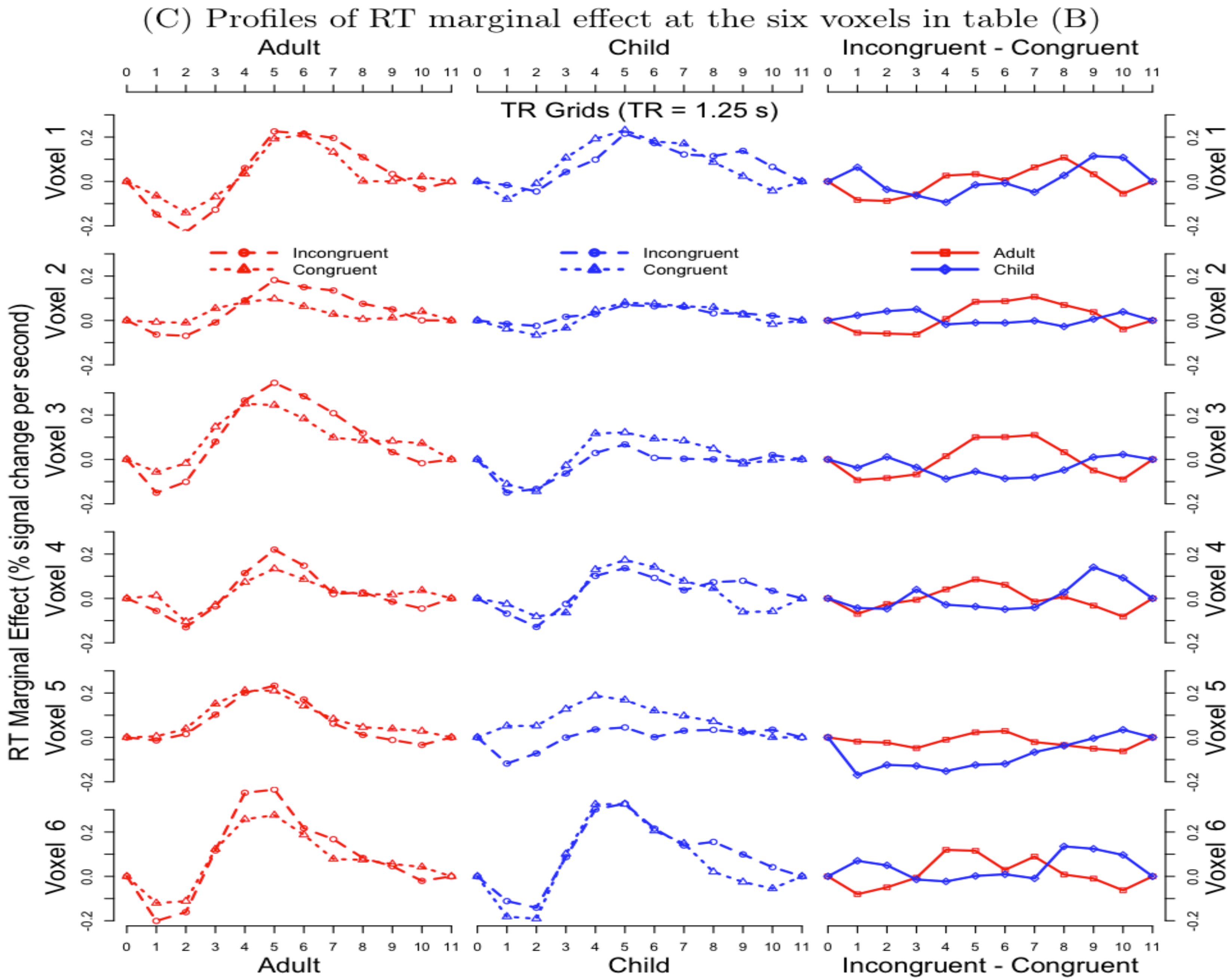
(A) Coronal view of interaction effect of Group:Condition:Time



(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, reproducibility) of signal: extent to which the levels of a factor are related to each other
  - Example – 3 sources of variability: conditions, sites, subjects
  - Traditional approach: random-effects ANOVAs
  - LME approach

$$\hat{\beta}_{ijk} = \alpha_0 + \alpha_1 * x_k + b_i + c_j + d_k + \epsilon_{ijk},$$
$$b_i \sim N(0, \tau_1^2), c_j \sim N(0, \tau_2^2), d_k \sim N(0, \tau_3^2), \epsilon_{ijk} \sim N(0, \sigma^2)$$

$$ICC_l = \frac{\tau_l^2}{\tau_l^2 + \tau_2^2 + \tau_3^2 + \sigma^2}, l = 1, 2, 3$$

  - **3dLME**

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			

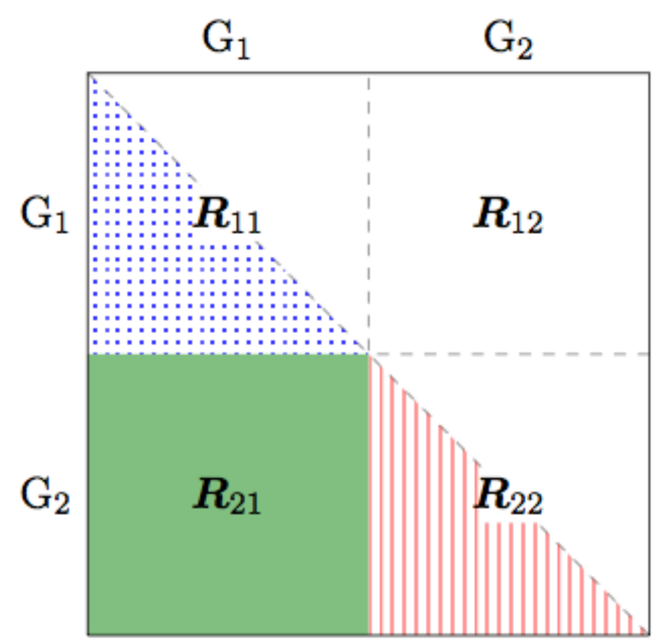
Inter-Subject Correlation (ISC)

- Conventional task-related FMRI experiments
  - Meticulously designed
  - Each trial lasts one or few TRs
  - Ultimate goal: identify ROIs associated with a task or a contrast
  - Potential issues: sensitivity (underpowered)
- **Naturalistic** tasks: lasting for a few minutes or more
  - Movie clip, music, speech
  - Minimally manipulated

Inter-Subject Correlation (ISC)

- Analysis methodology
  - Regression with task-related regressors won't work
  - Voxel-wise correlation between any subject pair with **`3dTcorrelate`**
    - $n = 4$ subjects → 6 ISC; $n = 5$ subjects → 10 ISC
    - n subjects → $n(n\text{-}1)/2$ ISC – which are not all independent!
  - How to go about group analysis?

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



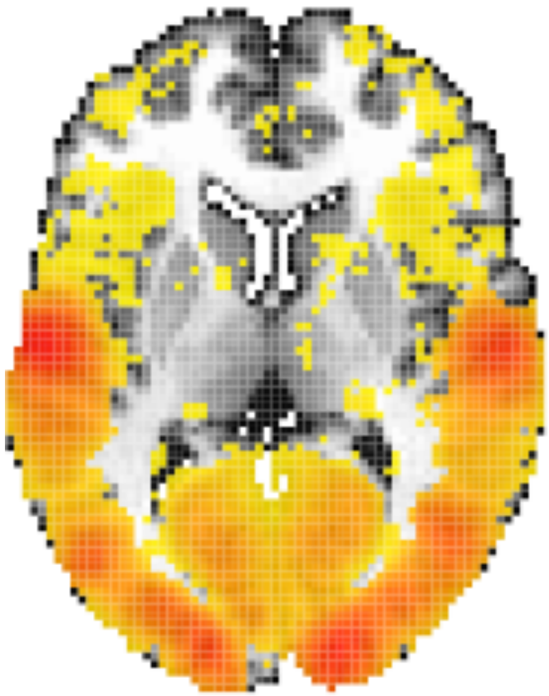
Inter-Subject Correlation (ISC)

- Analysis methodology
  - How to go about group analysis?
    - Difficulty: The ISCs are not independent with each other
    - The correlations are correlated themselves!

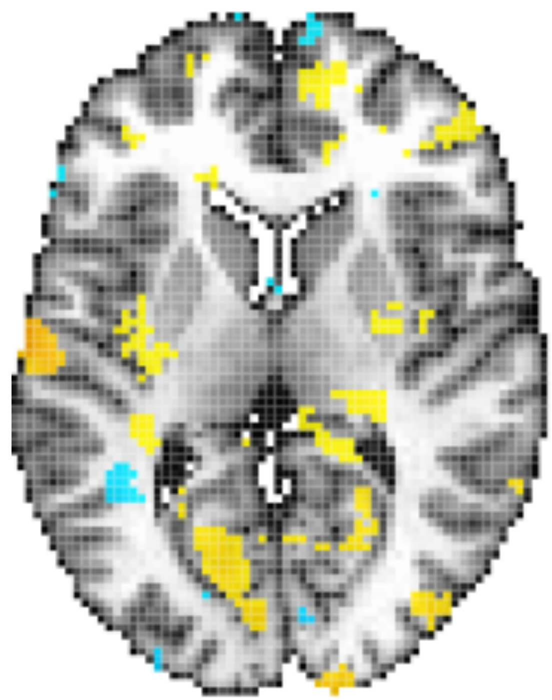
$$
\begin{array}{cc}
 & \begin{array}{cccccccccc} Z_{21} & Z_{31} & Z_{41} & Z_{51} & Z_{32} & Z_{42} & Z_{52} & Z_{43} & Z_{53} & Z_{54} \end{array} \\
\begin{array}{c} Z_{21} \\ Z_{31} \\ Z_{41} \\ Z_{51} \\ Z_{32} \\ Z_{42} \\ Z_{52} \\ Z_{43} \\ Z_{53} \\ Z_{54} \end{array} &
\begin{pmatrix}
1 & \rho & \rho & \rho & \rho & \rho & \rho & 0 & 0 & 0 \\
\rho & 1 & \rho & \rho & \rho & 0 & 0 & \rho & \rho & 0 \\
\rho & \rho & 1 & \rho & 0 & \rho & 0 & \rho & 0 & \rho \\
\rho & \rho & \rho & 1 & 0 & 0 & \rho & 0 & \rho & \rho \\
\rho & \rho & 0 & 0 & 1 & \rho & \rho & \rho & \rho & 0 \\
\rho & 0 & \rho & 0 & \rho & 1 & \rho & \rho & 0 & \rho \\
\rho & 0 & 0 & \rho & \rho & \rho & 1 & 0 & \rho & \rho \\
0 & \rho & \rho & 0 & \rho & \rho & 0 & 1 & \rho & \rho \\
0 & \rho & 0 & \rho & \rho & 0 & \rho & \rho & 1 & \rho \\
0 & 0 & \rho & \rho & 0 & \rho & \rho & \rho & \rho & 1
\end{pmatrix}
\end{array}
$$

Inter-Subject Correlation (ISC)

- Analysis methodology
  - How to go about group analysis?
    - Male group and difference between males and females



Males, $p < 0.001$



Males vs. Females, $p < 0.05$

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)
  - Nonparametric approach and fixed-effects analysis
- No routine statistical questions, only questionable routines!