

# Statistical Analysis

It is easy to lie with statistics. It is hard to tell the truth without it.

----[Andrejs Dunkels](#)

# FMRI Analysis

Experiment Design



Scanning



Pre-Processing



Individual Subject Analysis

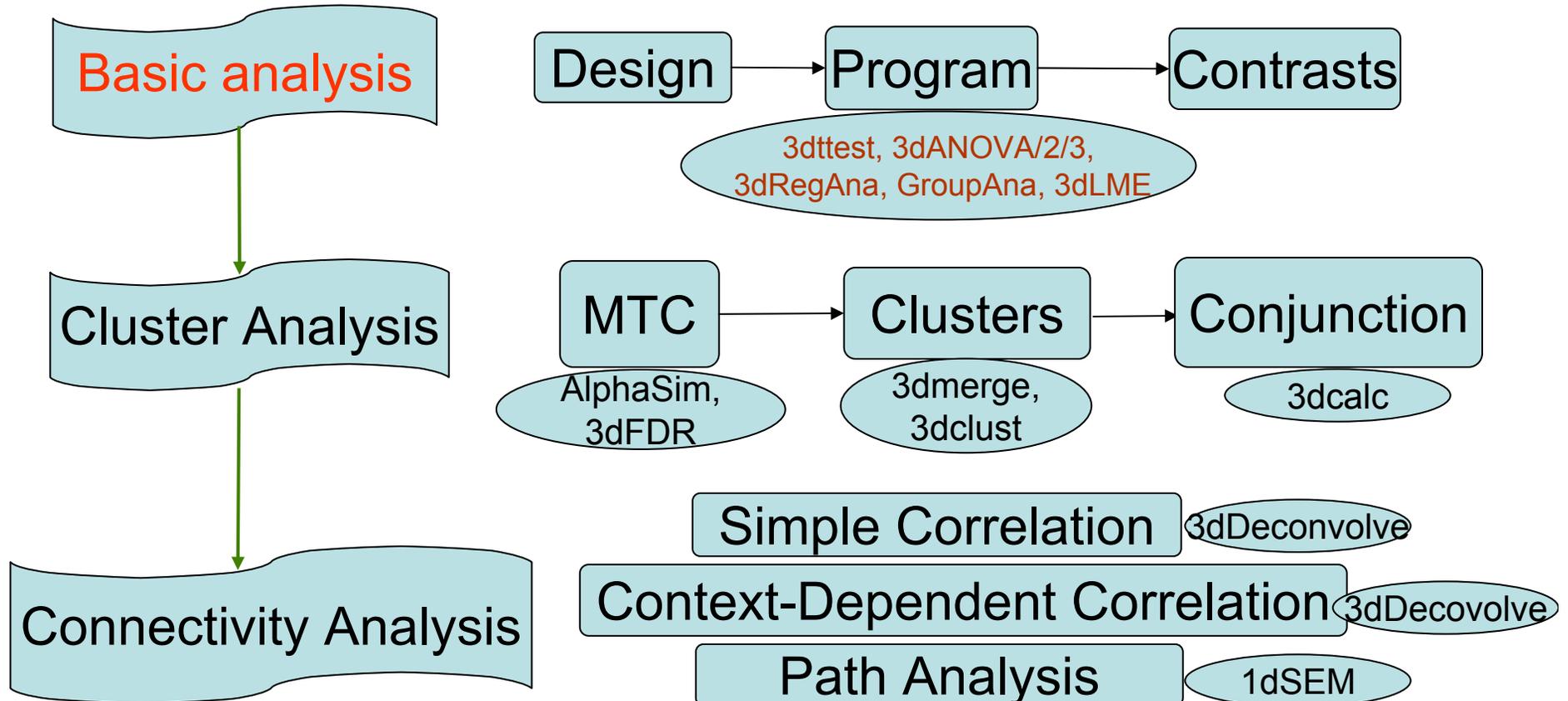


**Group Analysis**



Post-Processing

# Group Analysis



## • Group Analysis: Basic concepts

### 👉 Group analysis

- ↳ Make general conclusions about some population
- ↳ Partition/untangle data variability into various sources

### 👉 Why two tiers of analysis?

- ↳ High computation cost
- ↳ Within-subject variation relatively small compared to cross-subject

### 👉 Mess in terminology

- ↳ Fixed: factor, analysis/model/effects
  - Fixed-effects analysis (sometimes): averaging a few subjects
- ↳ Random: factor, analysis/model/effects
  - Random-effects analysis (sometimes): subject as a random factor  
But really a mixed-effects analysis
- ↳ Mixed: design, model/effects
  - Mixed design: crossed [e.g., AXBXC] and nested [e.g., BXC(A)]  
Psychologists: Within-subject (repeated measures) / between-subjects factor
  - Mixed-effects: model with both types of factors;  
model with both inter/intra-subject variances

- **Group Analysis: Basic concepts**

- ↳ **Fixed factor**

- ↳ Treated as a fixed variable in the model
  - Categorization of experiment conditions (mode: Face/House)
  - Group of subjects (male/female, normal/patient)
- ↳ All levels of the factor are of interest and included for all replications
- ↳ Fixed in the sense inferences
  - apply only to the specific levels of the factor
  - don't extend to other potential levels that might have been included

- ↳ **Random factor**

- ↳ Exclusively subject in FMRI
- ↳ Treated as a random variable in the model
  - average + random effects uniquely attributable to each subject:  $N(0, \sigma^2)$
- ↳ Each subject is of NO interest
- ↳ Random in the sense
  - subjects serve as a random sample of a population
  - inferences can be generalized to a population

## • Group Analysis: Types

### 👉 Averaging across subjects (fixed-effects analysis)

- ↙ Number of subjects  $n < 6$
- ↙ Case study: can't generalize to whole population
- ↙ Simple approach (3dca1c)
  - $T = \sum t_{ij} / \sqrt{n}$
- ↙ Sophisticated approach
  - $B = \sum (b_i / \sqrt{v_i}) / \sum (1 / \sqrt{v_i})$ ,  $T = B \sum (1 / \sqrt{v_i}) / \sqrt{n}$ ,  $v_i$  = variance for  $i$ -th regressor
  - $B = \sum (b_i / v_i) / \sum (1 / v_i)$ ,  $T = B \sqrt{[\sum (1 / v_i)]}$
  - Combine individual data and then run regression

### 👉 Mixed-effects analysis

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

- **Group Analysis: Programs in AFNI**

- ↳ **Non-parametric analysis**

- ↳ 4 < number of subjects < 10

- ↳ No assumption of normality; statistics based on ranking

- ↳ Programs

- `3dWilcoxon` (~ paired *t*-test)

- `3dMannWhitney` (~ two-sample *t*-test)

- `3dKruskalWallis` (~ between-subjects with `3dANOVA`)

- `3dFriedman` (~one-way within-subject with `3dANOVA2`)

- `Permutation test`

- : Multiple testing correction with FDR (`3dFDR`)

- ↳ Can't handle complicated designs

- ↳ Less sensitive to outliers (more robust) and less flexible than parametric tests

- **Group Analysis: Programs in AFNI**

- ↳ **Parametric tests (mixed-effects analysis)**

- ↳ Number of subjects > 10

- ↳ Assumption: Gaussian random effects

- ↳ Programs

- ↳ **3dttest** (one-sample, two-sample and paired t)

- ↳ **3dANOVA** (one-way between-subject)

- ↳ **3dANOVA2** (one-way within-subject, 2-way between-subjects)

- ↳ **3dANOVA3** (2-way within-subject and mixed, 3-way between-subjects)

- ↳ **3dRegAna** (regression/correlation, simple unbalanced ANOVA, simple ANCOVA)

- ↳ **GroupAna** (Matlab package for up to 5-way ANOVA)

- ↳ **3dLME** (R package for all sorts of group analysis)

## • Group Analysis: Planning

### 👉 How many subjects?

- ↳ Power/efficiency: proportional to  $\sqrt{n}$ ;  $n > 10$
- ↳ Balance: Equal number of subjects across groups if possible

### 👉 Input files

- ↳ Common brain in tlrc space (resolution doesn't have to be 1x1x1 mm<sup>3</sup>)
- ↳ % signal change (**not** statistics) or normalized variables
  - HRF magnitude: Regression coefficients
  - Contrasts

### 👉 Design

- ↳ Number of factors
- ↳ Number of levels for each factor
- ↳ Factor types
  - Fixed (factors of interest) vs. random (subject)
  - Cross/nesting: Balanced? Within-subject/repeated-measures vs. between-subjects
- ↳ Which program?
  - **3dttest, 3dANOVA/2/3, GroupAna, 3dRegAna, 3dLME**

# • Group Analysis: Planning

## 👉 Output

### ↳ Main effect $F$

- $F$ : general information about all levels of a factor
- Any difference response between two sexes

### ↳ Interaction $F$

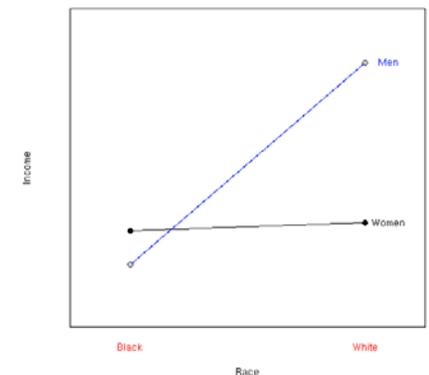
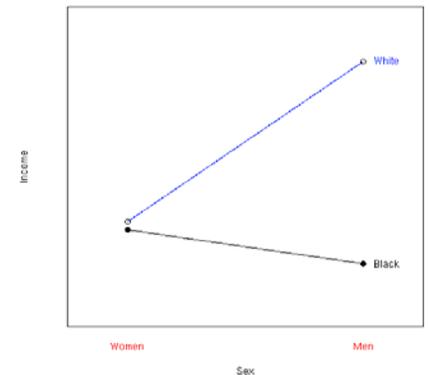
- Mutual/reciprocal influence among 2 or more factors
- Effect for each factor depends on levels of other factors

### ↳ General linear test

- Contrast
- General linear test (e.g., trend analysis)

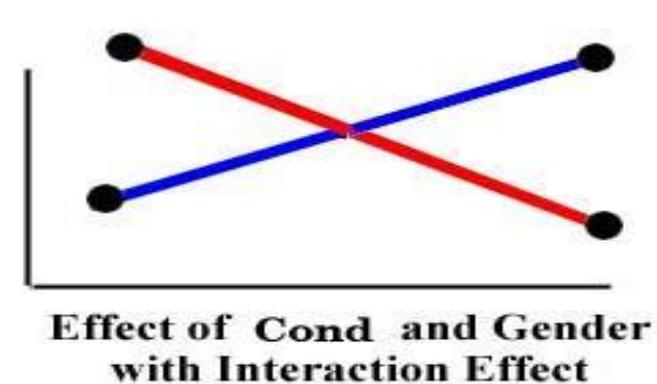
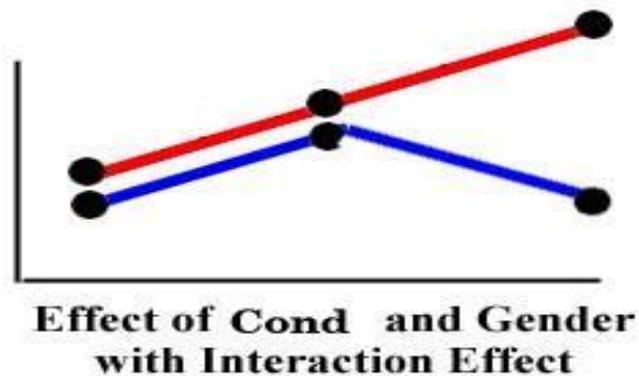
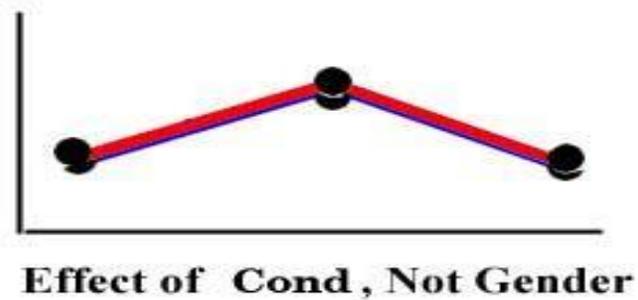
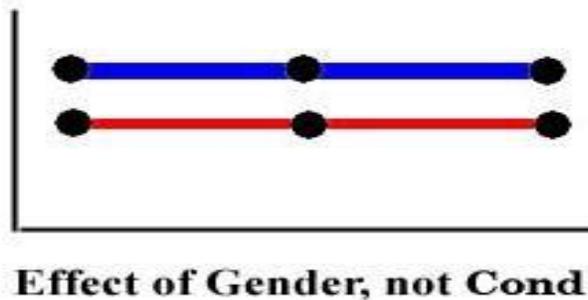
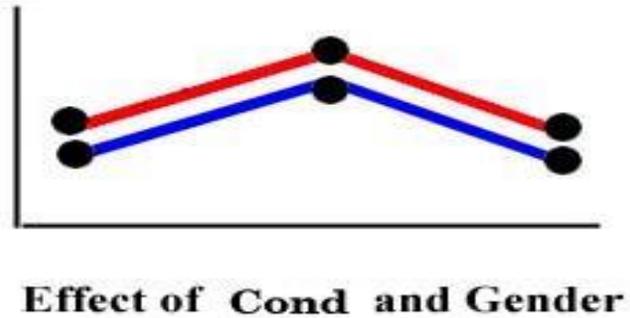
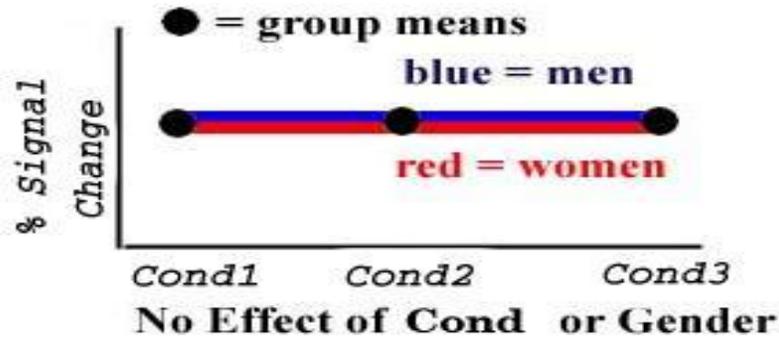
### ↳ Example

- Dependent variable: income
- Factor A: sex (men vs. women); factor B: race (whites vs. blacks)
- Main effects: men > women; whites > blacks
- Is it fair to only focus on main effects? Interaction!
  - Black men < black women;
  - Black women almost the same as white women;
  - Black men << white men



- Group Analysis: Main effect and interaction**

**Main effects and Interactions Between Gender and Condition**



# • Group Analysis: Planning

## 👉 Thresholding

- ↳ Two-tail by default in AFNI
- ↳ If one-tail  $p$  is desirable, look for  $2p$  on AFNI

## 👉 Scripting – 3dANOVA3

### ↳ **Three-way between-subjects** (type 1)

- 3 categorizations of groups: sex, disease, age

### ↳ **Two-way within-subject** (type 4): Crossed design AXBXC

- One group of subjects: 16 subjects
- Two categorizations of conditions: A – category; B - affect

### ↳ **Two-way mixed** (type 5): BXC(A)

- Nesting (between-subjects) factor (A): subject classification, e.g., sex
- One category of condition (within-subject factor B): condition (visual vs. auditory)
- Nesting: balanced

# • Group Analysis: Example

```
3dANOVA3 -type 4 -alevels 3 -blevels 3 -clevels 16 \  
-dset 1 1 1 stats.sb04.beta+tlrc'[0]' \  
-dset 1 2 1 stats.sb04.beta+tlrc'[1]' \  
-dset 1 3 1 stats.sb04.beta+tlrc'[2]' \  
-dset 2 1 1 stats.sb04.beta+tlrc'[4]' \  
...  
-fa Category \  
-fb Affect \  
-fab CatXAff \  
-amean 1 T \  
-acontr 1 0 -1 TvsF \  
-bcontr 0.5 0.5 -1 non-neu \  
-aBcontr 1 -1 0 : 1 TvsE-pos \  
-Abcontr 2 : 1 -1 0 HMvsHP \  
  
-bucket anova33
```

Model type,  
Factor levels

Input for each cell in  
ANOVA table:  
totally  $3 \times 3 \times 16 = 154$

F tests: Main effects &  
interaction

t tests: 1<sup>st</sup> order  
Contrasts

t tests: 2<sup>nd</sup> order  
Contrasts

Output: bundled

- **Group Analysis**: GroupAna

- 👉 **Multi-way ANOVA**

- ↙ Matlab script package for up to 5-way ANOVA
    - ↙ Can handle both volume and surface data
    - ↙ Can handle up to 4-way unbalanced designs
      - No missing data allowed
    - ↙ Downsides
      - Requires **Matlab** plus **Statistics Toolbox**
      - Slow: GLM approach - regression through dummy variables
      - Complicated design, and compromised power
    - ↙ Heavy duty computation
      - Minutes to hours
      - Input with lower resolution recommended
      - Resample with `adwarp -dxyz #` or `3dresample`
    - ↙ See <http://afni.nimh.nih.gov/sscc/gangc> for more info

- 👉 **Alternative: 3dLME**

- **Group Analysis**: ANCOVA (ANalysis of COVAriances)

- 👉 **Why ANCOVA?**

- ↯ Subjects or cross-regressors effects might not be an ideally randomized
    - ↯ If not controlled, such variability will lead to loss of power and accuracy
    - ↯ Different from **amplitude modulation (AM)**: **cross**-regressor vs. **within**-regressor variation
    - ↯ Direct control through experiment design: balanced selection of subjects (e.g., age group)
    - ↯ Indirect (statistical) control: add covariates in the model
    - ↯ Covariate (variable of no interest): uncontrollable/confounding, usually continuous
      - Age, IQ, cortex thickness
      - Behavioral data, e.g., response time, correct/incorrect rate, symptomatology score, ...

- 👉 **ANCOVA = Regression + ANOVA**

- ↯ Assumption: **linear** relation between HDR and the covariate
    - ↯ GLM approach: accommodate both categorical and quantitative variables

- 👉 **Programs**

- ↯ **3dRegAna**: for simple ANCOVA
      - If the analysis can be handled with 3dtttest without covariates
    - ↯ **3dLME**: R package (versatile)

## • Group Analysis: ANCOVA Example

### 👉 Example: Running ANCOVA

↪ Two groups: 15 normal vs. 13 patients

↪ Analysis

➤ Compare two group: without covariates, two-sample  $t$  with `3dtttest`

➤ Controlling age effect

↪ GLM model

➤  $Y_i = \beta_0 + \beta_1 X_{1i} + \beta_2 X_{2i} + \beta_3 X_{3i} + \varepsilon_i, i = 1, 2, \dots, n (n = 28)$

➤ Code the factor (group) with dummy coding

0, when the subject is a patient – control/reference group;

$X_{2i} = \{$

1, when the subject is normal.

➤ **Centralize** covariate (age)  $X_1$  so that

$\beta_0$  = patient effect;  $\beta_1$  = age effect (correlation coef);  $\beta_2$  = normal vs patient

➤  $X_{3i} = X_{1i} X_{2i}$  models interaction (optional) between covariate and factor (group)

$\beta_3$  = interaction

## • Group Analysis: ANCOVA Example

```
3dRegAna -rows 28 -cols 3 \  
-xydata 0.1 0 0 patient/Pat1+tlrc.BRIK \  
-xydata 7.1 0 0 patient/Pat2+tlrc.BRIK \  
...  
-xydata 7.1 0 0 patient/Pat13+tlrc.BRIK \  
-xydata 2.1 1 2.1 normal/Norm1+tlrc.BRIK \  
-xydata 2.1 1 2.1 normal/Norm2+tlrc.BRIK \  
...  
-xydata 0.1 1 0.1 normal/Norm15+tlrc.BRIK \  
-model 1 2 3 : 0 \  
-bucket 0 Pat_vs_Norm \  
-brick 0 coef 0 'Pat' \  
-brick 1 tstat 0 'Pat t' \  
-brick 2 coef 1 'Age Effect' \  
-brick 3 tstat 1 'Age Effect t' \  
-brick 4 coef 2 'Norm-Pat' \  
-brick 5 tstat 2 'Norm-Pat t' \  
-brick 6 coef 3 'Interaction' \  
-brick 7 tstat 3 'Interaction t'
```

Model parameters: 28 subjects,  
3 independent variables

Input: Covariates, factor levels,  
interaction, and input files

Specify model for F and R<sup>2</sup>

Output: #subbricks = 2\*#coef + F + R<sup>2</sup>

Label output subbricks  
for  $\beta_0, \beta_1, \beta_2, \beta_3$

See <http://afni.nimh.nih.gov/sscc/gangc/ANCOVA.html> for more information

# • Group Analysis: 3dLME

## 👉 An R package

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

## • Group Analysis: 3dLME

### ↳ Linear model

$$\leftarrow y_i = \beta_0 + \beta_1 x_{1i} + \dots + \beta_p x_{pi} + \varepsilon_i, \varepsilon_i \sim \text{NID}(0, \sigma^2)$$

$$\leftarrow Y = X\beta + \varepsilon, \varepsilon \sim N_n(0, \sigma^2 I_n)$$

↳ Only one random effect, residual  $\varepsilon$

### ↳ Linear mixed-effects (LME) model

$$\leftarrow y_{ij} = \beta_0 + \beta_1 x_{1ij} + \dots + \beta_p x_{pij} + b_{i1} z_{1ij} + \dots + b_{iq} z_{qij} + \varepsilon_{ij},$$

$$\leftarrow b_{ik} \sim N(0, \psi_k^2), \text{cov}(b_k, b_{k'}) = \psi_{kk'}, \varepsilon_{ij} \sim N(0, \sigma^2 \lambda_{ijj}), \text{cov}(\varepsilon_{ij}, \varepsilon_{ij'}) = \sigma^2 \lambda_{ijj},$$

$$\leftarrow Y_i = X_i \beta + Z_i b_i + \varepsilon_i, b_i \sim N_q(0, \psi), \varepsilon_i \sim N_{n_i}(0, \sigma^2 \Lambda_i)$$

↳ Two random effect components:  $Z_i b_i$  and  $\varepsilon_i$

↳ In fMRI, usually  $q=1$ ,  $Z_i = I_{n_i}$  – subject: one parameter  $\psi$

## • Group Analysis: 3dLME

### ↳ Linear mixed-effects (LME) model

↳ For each subject  $Y_i = X_i\beta + Z_i b_i + \varepsilon_i$ ,  $b_i \sim N_q(0, \psi)$ ,  $\varepsilon_i \sim N_{n_i}(0, \sigma^2 \Lambda_i)$

↳ AN(C)OVA can be incorporated as a special case

➤  $n_i$  is constant ( $>1$ , repeated-measures),  $\Lambda_i = I_{n \times n}$  (iid)

↳ LME is much more flexible

➤ No differentiation between categorical and continuous variables (ANOVA vs. ANCOVA)

➤  $n_i$  can vary (unbalanced design: unequal number of subjects, missing data)

➤ Don't have to include an intercept: basis functions!

➤ Residual variance-covariance  $\sigma^2 \Lambda_i$  can be any structure

## • Group Analysis: 3dLME

✎ LME: correlation structure in  $\sigma^2\Lambda_i$  - off-diagonals

- iid  $\Lambda_i = I_{n \times n}$ : traditional AN(C)OVA; one parameter  $\sigma^2$
- Compound symmetry: 2 parameters  $\sigma^2$  and  $\sigma_1$

$$\sigma^2\Lambda_i = \begin{pmatrix} \sigma^2 + \sigma_1 & \sigma_1 & \dots & \sigma_1 \\ \sigma_1 & \sigma^2 + \sigma_1 & \dots & \sigma_1 \\ \vdots & \vdots & \ddots & \vdots \\ \sigma_1 & \sigma_1 & \dots & \sigma^2 + \sigma_1 \end{pmatrix}$$

Assume equal correlation across factor levels: fixed variance/covariance

- First-order autoregressive structure AR(1): 2 parameters  $\sigma^2$  and  $\rho$

$$\sigma^2\Lambda_i = \begin{pmatrix} \sigma^2 & \sigma^2\rho & \dots & \sigma^2\rho^{n_i-1} \\ \sigma^2\rho & \sigma^2 & \dots & \sigma^2\rho^{n_i-2} \\ \vdots & \vdots & \ddots & \vdots \\ \sigma^2\rho^{n_i-1} & \sigma^2\rho^{n_i-2} & \dots & \sigma^2 \end{pmatrix}$$

Equally-spaced longitudinal observations across factor levels

- ARMA( $p, q$ ):  $p+q$  parameters

- Group Analysis: 3dLME

- ☞ LME: variance structure in  $\sigma^2\Lambda_j$  - diagonals

- iid  $\Lambda_j = I_{n \times n}$ : traditional AN(C)OVA; one parameter  $\sigma^2$

- Heteroscedasticity: different  $\sigma^2$  across factor levels;  $n_j+1$  parameters

- ☞ HRF modeled with basis functions

- Traditional approach: AUC

- Can't detect shape difference

- Difficult to handle betas with mixed signs

- LME approach

- Usually  $H_0: \beta_1 = \beta_2 = \dots = \beta_k$

- But now we don't care about the differences among  $\beta$ s

- $H_0: \beta_1 = \beta_2 = \dots = \beta_k = 0$

- Solution: take all  $\beta$ s and model with no intercept

- But we have to deal with temporal correlations among  $\beta$ s!

# • Group Analysis: 3dLME

## 👉 Running LME

↳ <http://afni.nimh.nih.gov/sscc/gangc/lme.html>

↳ Install 3dLME.R and a few packages

↳ Create a text file `model.txt` (3 fixed factors plus 1 covariate)

```
DataFormat          <-- either Volume or Surface
OutputFileName      <-- any string (no suffix needed)
MASK:Mask+tlrc.BRIK <-- mask dataset
Gender*Object*Modality+Age <-- model formula for fixed effects
COV:Age             <-- covariate list
SavedForRandomEffects <-- space reserved for future
MFace-FFace        <-- contrast label
Male*Face*0*0-Female*Face*0*0 <-- contrast specification
MVisual-Maudial
Male*0*Visual*0-Male*0*Audial*0
```

.....

Subj	Gender	Object	Modality	Age	InputFile
Jim	Male	Face	Visual	25	file1+tlrc.BRIK
Carol	Female	House	Audial	23	file2+tlrc.BRIK
Karl	Male	House	Visual	26	file3+tlrc.BRIK
Casey	Female	Face	Audial	24	file4+tlrc.BRIK

.....

↳ Run R CMD BATCH \$LME/3dLME.R MyOut &

# • Group Analysis: 3dLME

👉 Running LME: A more complicated example (still testing)

↳ HRF modeled with 6 tents

↳ Null hypothesis: no HRF difference between two conditions

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

Subj    Time    TimeOrder  InputFile
Jim     t1      1    contrastT1+tlrc.BRIK
Jim     t2      2    contrastT2+tlrc.BRIK
Jim     t3      3    contrast3+tlrc.BRIK
Jim     t4      4    contrast4+tlrc.BRIK
.....
```

# • Group Analysis: 3dLME

## ☞ Running LME: model fine-tuning (planning)

### ↙ How to specify 4 structures:

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

### ↙ Pick up a most interesting voxel

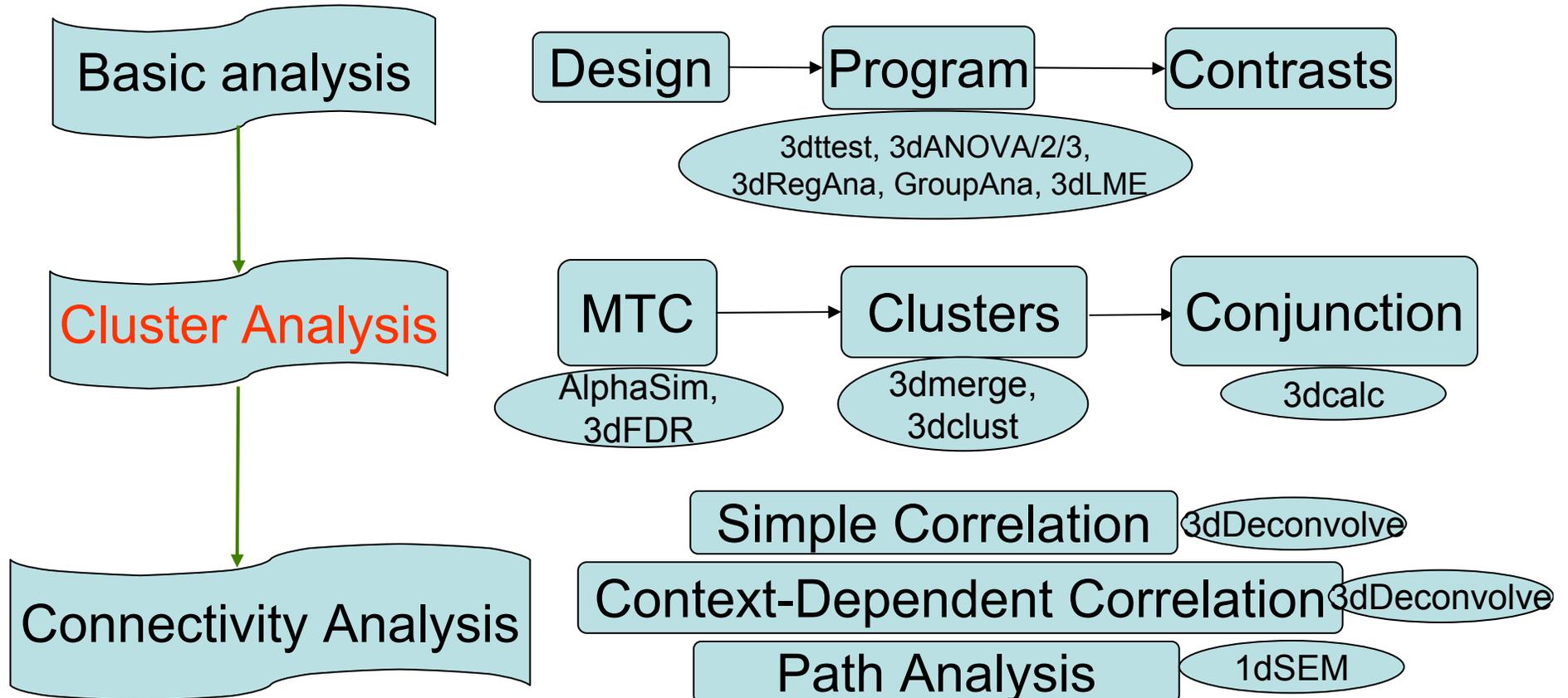
### ↙ Start with a reasonably simple model, and compare alternatives

- Add or reduce fixed and random effects
- Vary variance and correlation structures

### ↙ Problems

- The best model at one voxel might not be true for other voxels
- More sophisticated model means more parameters and longer running time
- Solution: ROI analysis – analyze each ROI separately!

# Group Analysis



- **Cluster Analysis: Multiple testing correction**

- ☞ **Two types of errors**

- ☞ What is  $H_0$  in fMRI studies?  $H_0$ : no effect (activation, difference, ...) at a voxel

- ☞ Type I =  $P(\text{reject } H_0 | \text{when } H_0 \text{ is true}) = \text{false positive} = p \text{ value}$

- Type II =  $P(\text{accept } H_0 | \text{when } H_1 \text{ is true}) = \text{false negative} = \beta$

- power =  $1 - \beta = \text{probability of detecting true activation}$

- ☞ Strategy: controlling type I error while increasing power (decreasing type II)

- ☞ Significance level  $\alpha$  (magic number 0.05) :  $p < \alpha$

## Justice System: Trial

## Statistics: Hypothesis Test

		Hidden Truth	
		Defendant Innocent	Defendant Guilty
Reject Presumption of Innocence (Guilty Verdict)	Type I Error	Correct	
	Fail to Reject Presumption of Innocence (Not Guilty Verdict)	Correct	Type II Error

		Hidden Truth	
		$H_0$ True Not Activated	$H_0$ False Activated
Reject $H_0$ (Activation decision)	Type I Error	Correct	
	Fail to Reject $H_0$ (No Activation decision)	Correct	Type II Error

# • Cluster Analysis: Multiple testing correction

## 👉 Family-Wise Error (FWE)

↳ Birth rate  $H_0$ : sex ratio at birth = 1:1

➤ What is the chance there are 5 boys (or girls) in a family?  $(1/2)^5 \sim 0.03$

➤ In a pool of 10000 families with 5 kids, expected #families with 5 boys =?  
 $10000 \times (1/2)^5 \sim 300$

↳ Multiple testing problem: voxel-wise statistical analysis

➤ With  $n$  voxels, what is the chance to mistake  $\geq$  one voxel?

Family-Wise Error:  $\alpha_{FW} = 1 - (1 - p)^n \rightarrow 1$  as  $n$  increases

➤  $n \sim 20,000$  voxels in the brain

## 👉 Multiple testing problem in FMRI

↳ 3 occurrences of multiple tests: individual, group, and conjunction

↳ Group analysis is the most severe one

# • Cluster Analysis: Multiple testing correction

## 👉 Approaches

### ↳ Control FWE

- Overall significance:  $\alpha_{FW} = P(\geq \text{one false positive voxel in the whole brain})$
- Bonferroni correction:  $\alpha_{FW} = 1 - (1 - p)^n \sim np$ , if  $p \ll 1/n$ 
  - \* Use  $p = \alpha/n$  as individual voxel significance level to achieve  $\alpha_{FW} = \alpha$
  - \* Too stringent and overly conservative:  $p = 10^{-8} \sim 10^{-6}$
- Something to rescue?
  - \* Correlation: Voxels in the brain are not independent
  - \* Cluster: Structures in the brain
  - \* Control FWE based on spatial correlation and cluster size

### ↳ Control false discovery rate (FDR)

- FDR = expected proportion of false + voxels among all [detected](#) voxels

## • Cluster Analysis: AlphaSim

### 👉 FWE in AFNI

↳ Monte Carlo simulations with **AlphaSim**

↳ Named for Monte Carlo, Monaco, where the primary attractions are casinos

↳ Program: **AlphaSim**

➤ Randomly generate some number (e.g., 1000) of brains with white noise

➤ Count the proportion of voxels are false + in **ALL** (e.g., 1000) brains

➤ Parameters:

\* ROI - mask

\* Spatial correlation FWHM: 3dBlurToFWHM or 3dFWHM!!

\* Connectivity – radius: how to identify voxels belong to a cluster?

\* Individual voxel significant level - uncorrected  $p$

➤ Output

\* Simulated (estimated) **overall significance level** (corrected  $p$ -value)

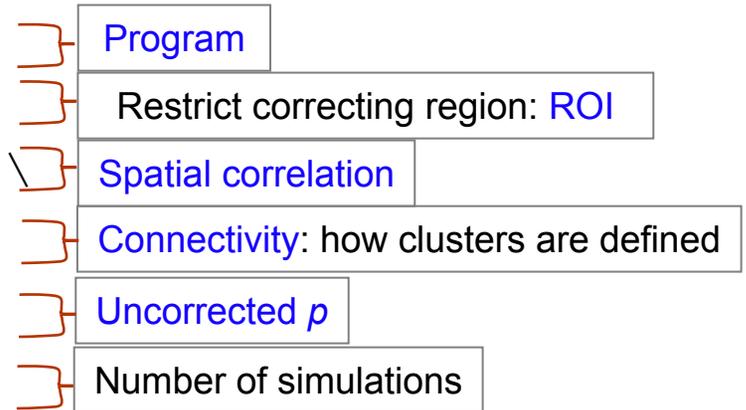
\* Corresponding **minimum cluster size**

- Cluster Analysis: AlphaSim

↳ Program: AlphaSim

➤ Example

```
AlphaSim \
-mask MyMask+orig \
-fwhmx 8.5 -fwhmy 7.5 -fwhmz 8.2 \
-rmm 6.3 \
-pthr 0.0001 \
-iter 1000
```



➤ Output: 5 columns

- \* Focus on the 1<sup>st</sup> and last columns, and ignore others
- \* 1<sup>st</sup> column: minimum cluster size in voxels
- \* Last column: alpha ( $\alpha$ ), overall significance level (corrected  $p$  value)

Cl Size	Frequency	Cum Prop	p/Voxel	Max Freq	Alpha
2	1226	0.999152	0.00509459	831	0.859
5	25	0.998382	0.00015946	25	0.137
10	3	1.0	0.00002432	3	0.03

➤ May have to run several times with different uncorrected  $p$

uncorrected  $p \uparrow \leftrightarrow$  cluster size  $\uparrow$

➤ See detailed steps at <http://afni.nimh.nih.gov/sscc/gangc/mcc.html>

# • Cluster Analysis: 3dFDR

## 👉 Definition

FDR = % false + voxels among all detected voxels in ONE brain

$$FDR = \frac{N_{ia}}{D_a} = \frac{N_{ia}}{N_{ia} + N_{aa}}$$

- ↳ FDR only focuses on individual voxel's significance level within the ROI, but doesn't consider any spatial structure
  - spatial correlation
  - cluster size

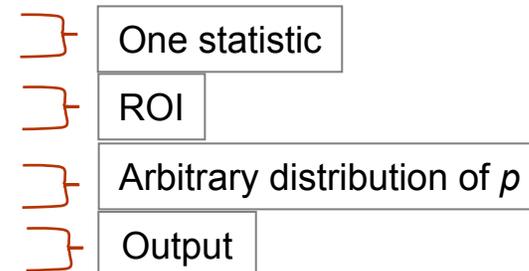
	Declared Inactive	Declared Active	
Truly Inactive	$N_{ii}$	$N_{ia} (I)$	$T_i$
Truly Active	$N_{ai} (II)$	$N_{aa}$	$T_a$
	$D_i$	$D_a$	

## 👉 Algorithm

- ↳ statistic ( $t$ ) →  $p$  value → FDR ( $q$  value) →  $z$  score
- ↳ Automatically calculated in most statistical programs in AFNI
- ↳ Automatically shown on AFNI viewer

## 👉 Example

```
3dFDR -input 'Group+tlrc[6]' \
      -mask_file mask+tlrc \
      -cdep -list \
      -output test
```



## • Cluster Analysis: FWE or FDR?

### 👉 FWE or FDR? Correct type I error in different sense

↳ FWE:  $\alpha_{FW} = P(\geq \text{one false positive voxel in the whole brain})$

- Frequentist's perspective: Probability among **many** hypothetical activation brains
- Used usually for parametric testing (Gaussian assumption)

↳ FDR = expected % false + voxels among all detected voxels

- Focus: controlling false + among detected voxels in **one** brain
- More frequently used in non-parametric testing (no Gaussian assumption)

↳ Concrete example

- Individual voxel  $p = 0.001$  for a brain of 25,000 EPI voxels
- Uncorrected → 25 false + voxels in the brain
- FWE: corrected  $p = 0.05$  → 5% false + hypothetical brains for a **fixed** voxel location
- FDR:  $q = 0.05$  → 5% voxels in those **positively** labeled ones are false +

### 👉 Fail to survive correction?

↳ Tricks

- One-tail?
- ROI – e.g., grey matter or whatever ROI you planned to look into

↳ Analysis on surface

- **Cluster Analysis**: Conjunction analysis

- ↳ **Conjunction analysis**

- ↳ Common activation area: intersection

- ↳ Exclusive activations

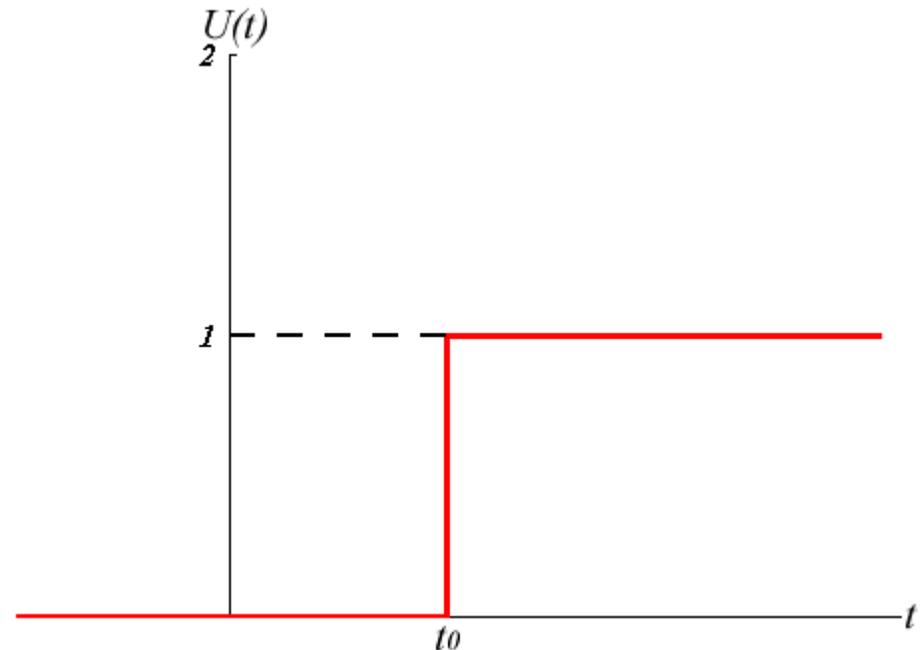
- ↳ With  $n$  entities, we have  $2^n$  possibilities (review your combinatorics!)

- ↳ **Tool: 3dcalc**

- ↳ Heaviside unit (**step function**)

- defines a *On/Off* event

$$U(t - t_0) = \begin{cases} 1 & t \geq t_0 \\ 0 & t < t_0 \end{cases}$$



- **Cluster Analysis**: Conjunction analysis

- ↳ **Example**

- ↳ 3 contrasts A, B, and C

- ↳ Assign each based on binary system: A: 001( $2^0=1$ ); B: 010( $2^1=2$ ); C: 100( $2^2=4$ )

- ↳ Create a mask with 3 sub-bricks of  $t$  (e.g., threshold = 4.2)

- ```
3dcalc -a ContrA+tlrc -b ContrB+tlrc -c ContrC+tlrc \  
-expr '1*step(a-4.2)+2*step(b-4.2)+4*step(c-4.2)' \  
-prefix ConjAna
```

- ↳ Interpret output - 8 ( $=2^3$ ) scenarios:

- 000(0): none;

- 001(1): A but no others;

- 010(2): B but no others;

- 011(3): A and B but not C;

- 100(4): C but no others;

- 101(5): A and C but not B;

- 110(6): B and C but not A;

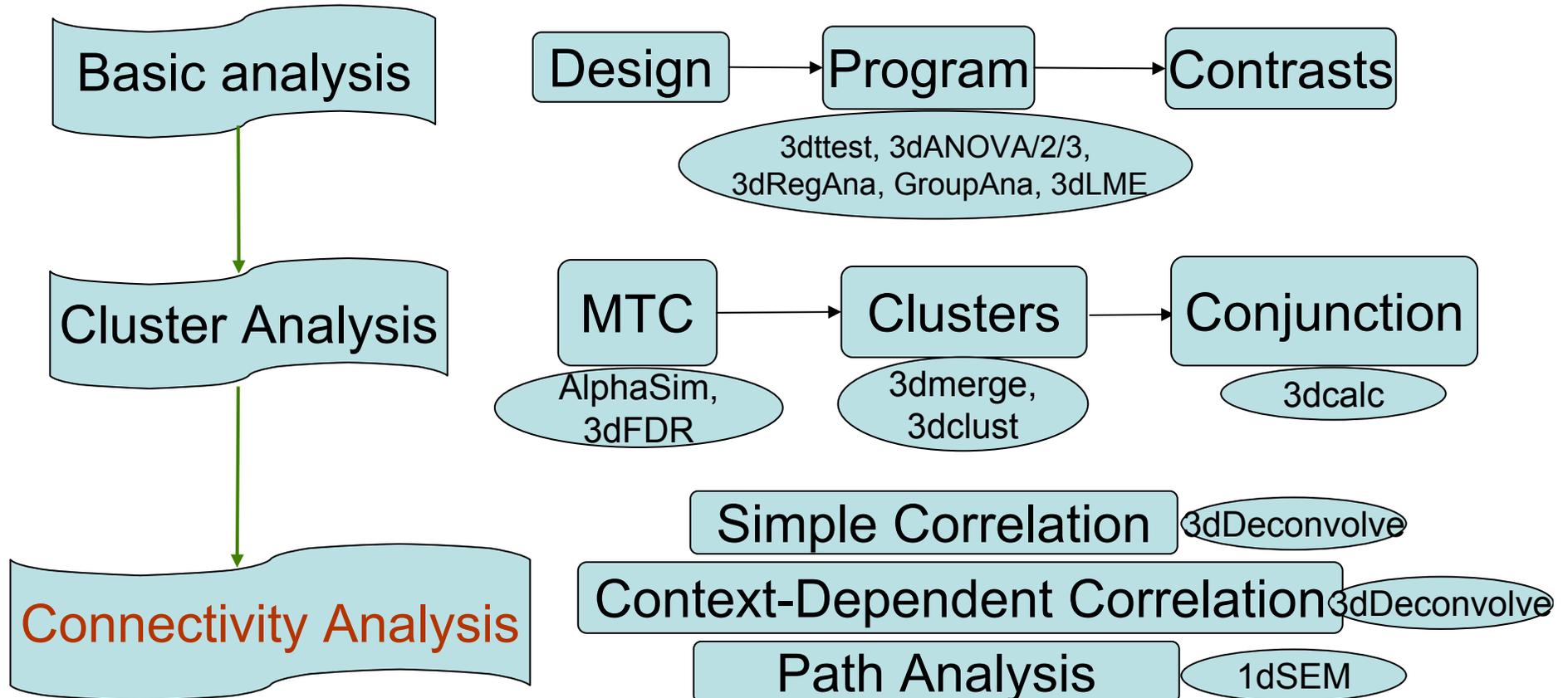
- 111(7): A, B and C

- **Cluster Analysis**: Conjunction analysis

- **Multiple testing correction issue**

- How to calculate the  $p$ -value for the conjunction map?
    - No problem if each entity was corrected before conjunction analysis
    - But that may be too stringent (conservative) and over-corrected
    - With 2 or 3 entities, analytical calculation possible
      - Each can have different uncorrected  $p$
      - Double or triple integral of Gaussian distributions
    - With more than 3 entities, may have to resort to simulations
      - Monte Carlo simulations
      - A program in the pipeline?

# Group Analysis



## • Connectivity: Correlation Analysis

### 👉 Correlation analysis

- ↯ Similarity between a seed region and the rest of the brain
- ↯ Says not much about causality/directionality
- ↯ Voxel-wise analysis
- ↯ Both individual subject and group levels
- ↯ Two types: **simple** and **context-dependent** correlation (a.k.a. PPI)

### 👉 Steps at individual subject level

- ↯ Create ROI
- ↯ Isolate signal for a condition/task
- ↯ Extract seed time series
- ↯ Correlation analysis through regression analysis
- ↯ More accurately, partial (multiple) correlation

### 👉 Steps at group level

- ↯ Convert correlation coefficients to Z (Fisher transformation): `3dcalc`
- ↯ One-sample *t* test on Z scores: `3dttest`

👉 **More details:** <http://afni.nimh.nih.gov/sscc/gangc>

# • Connectivity: Path Analysis or SEM

## 👉 Causal modeling (a.k.a. structural connectivity)

↳ Start with a network of ROI's

↳ Path analysis

➤ Assess the network based on correlations (covariances) of ROI's

➤ Minimize discrepancies between correlations based on data and estimated from model

➤ Input: Model specification, correlation matrix, residual error variances, DF

➤ Output: Path coefficients, various fit indices

↳ Caveats

➤  $H_0$ : It is a good model

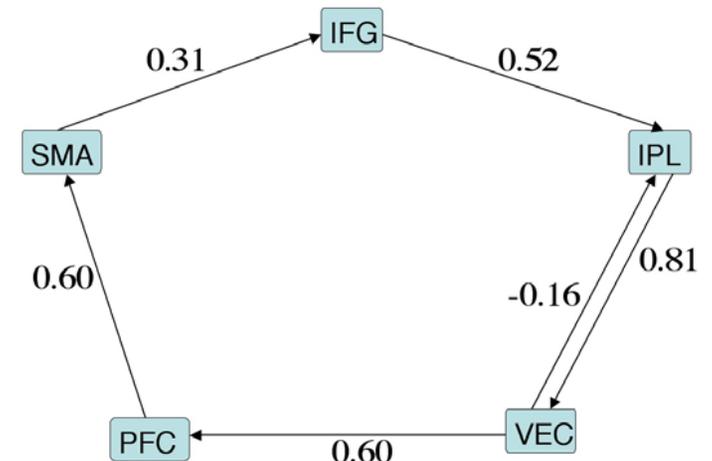
➤ Valid only with the data and model specified

➤ No proof: modeled through correlation analysis

➤ Even with the same data, an alternative model might be equally good or better

➤ If one critical ROI is left out, things may go awry

➤ Interpretation of path coefficient



# • Connectivity: Path Analysis or SEM

## 👉 Path analysis with 1dSEM

- ↳ Model validation: 'confirm' a theoretical model
  - Accept, reject, or modify the model?
- ↳ Model search: look for 'best' model
  - Start with a minimum model (1): can be empty
  - Some paths can be excluded (0), and some optional (2)
  - Model grows by adding one extra path a time
  - 'Best' in terms of various fit criteria
- ↳ More information <http://afni.nimh.nih.gov/sscc/gangc/PathAna.html>

## 👉 Difference between causal and correlation analysis

- ↳ Predefined network (model-based) vs. network search (data-based)
- ↳ Modeling: causation (and directionality) vs. correlation
- ↳ ROI vs. voxel-wise
- ↳ Input: correlation (condensed) vs. original time series
- ↳ Group analysis vs. individual + group