FMRI Analysis

Experiment Design

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Scanning

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Pre-Processing

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Individual Subject Analysis

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Group Analysis

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Post-Processing

Group Analysis

Basic analysis → Design → Program → Contrasts

3dttest, 3dANOVA/2/3, 3dRegAna, GroupAna, 3dLME

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Cluster Analysis → MTC → Clusters → Conjunction

AlphaSim, 3dFDR

3dmerge, 3dclust

3dcalc

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Connectivity Analysis

Simple Correlation

Context-Dependent Correlation

Path Analysis

- **Group Analysis: Basic concepts**
  - Group analysis
    - Make general conclusions about some population
    - Partition/untangle data variability into various sources
  - Why two ties of analysis?
    - High computation cost
    - Within-subject variation ignored
  - Mess in terminology
    - Fixed: factor, model (effects)
      - Fixed-effects analysis (sometimes): averaging a few subjects
    - Random: factor, 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

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- **Group Analysis: Basic concepts**
  - Fixed factor
    - Treated as a fixed variable in the model
      - Categorization of experiment conditions (modality: visual/auditory)
      - Group of subjects (gender, normal/patients)
    - All levels of the factor are of interest and included for all replications
    - Fixed in the sense inferences
      - apply only to the specific levels of the factor
      - don't extend to other potential levels that might have been included
  - Random factor
    - Exclusively subject in FMRI
    - Treated as a random variable in the model
      - average + random effects uniquely attributable to each subject: $N(\mu, \sigma^2)$
    - 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

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- **Group Analysis: Types**
  - Averaging across subjects
    - Number of subjects $n < 6$
    - Case study: can't generalize to whole population
    - Simple approach (**`3dcalc`**)
      - $T = \sum t_{ii}/\sqrt{n}$
    - 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

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- **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`**plugin on AFNI under Define Datamode / Plugins /;

        C program by Tom Holroyd

        : Multiple testing correction with FDR (**`3dFDR`**)
    - Can't handle complicated designs
    - Less sensitive to outliers (more robust) and less flexible than parametric tests

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- **Group Analysis: Programs in AFNI**
  - Parametric tests
    - Number of subjects > 10
    - Assumption: Gaussian
    - 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, hi-way or unbalanced ANOVA, ANCOVA)
      - **`GroupAna`** (Matlab package for up to 5-way ANOVA)
      - **`3dLME`** (R package for all sorts of group analysis)

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- **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^3)
    - % 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`**, **`RegAna`**, **`3dLME`**

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- **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
    - 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

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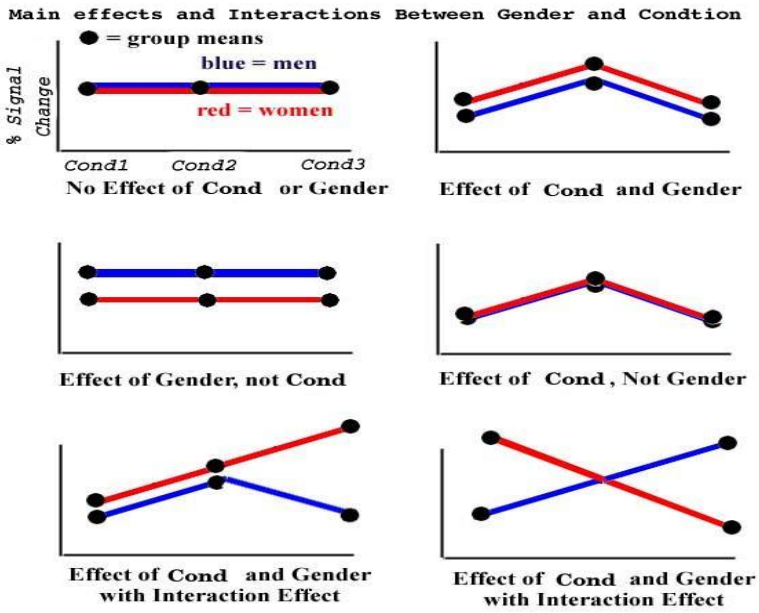
- **Group Analysis: Main effect and interaction**



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- **Group Analysis: Planning**
  - Output
    - General linear test
      - Contrast
      - General linear test
      - Trend analysis
  - Thresholding
    - Two-tail by default
    - For a desirable one-tail *p*, look for 2*p* 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    \ (coding with indices)
-acontr   1 0 -1 TvsF \ (coding with coefficients)
-bcontr   0.5 0.5 -1 non-neu \ (coefficients)
-aBcontr  1 -1 0 : 1 TvsE-pos \ (coefficients)
-Abcontr  2 : 1 -1 0 HMvsHP \ (coefficients)

-bucket anova33
```

Model type, Factor levels

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

F tests: Main effects & interaction

t tests: 1st order Contrasts

t tests: 2nd order Contrasts

Output: bundled

- **Group Analysis**: GroupAna
  - Multi-way ANOVA
    - Matlab script package for up to 5-way ANOVA
    - 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`

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- **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-regressors 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, …
      - Even categorical factors such as gender
  - 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 3dttest without covariates
    - **3dLME**: R package (versatile)

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- **Group Analysis**: ANCOVA Example
  - Example: Running ANCOVA
    - Two groups: 15 normal vs. 13 patients
    - Analysis
      - Compare two group: without covariates, two-sample *t* with **`3dttest`**
      - Controlling age effect
    - GLM model
      - $Y_i = \beta_0 + \beta_1 X_{1i} + \beta_2 X_{2i} + \beta_3 X_{3i} + \varepsilon_i$, $i = 1, 2, \ldots, n$ ($n = 28$)
      - Code the factor (group) with dummy coding

$$X_{2i} = \begin{cases} 0, & \text{when the subject is a patient – control/reference group;} \\ 1, & \text{when the subject is normal.} \end{cases}$$

      - **Centralize** covariate (age) X_1 so that

        β_0 = patient effect; β_1 = age effect (correlation coef); β_2 = normal vs patient
      - $X_{3i} = X_{1i} X_{2i}$ models interaction (optional) between covariate and factor (group)

        β_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^2

Output: #subbriks = 2*#coef + F + R^2

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 modeling (LME)
    - Versatile: handles almost all situations in one package
      - Unbalanced designs
      - Missing data
      - ANOVA and ANCOVA
      - Modeling with basis functions
      - Heteroscedasticity, variance-covariance structure
    - No scripting
    - Disadvantages
      - High computation cost
      - Sometimes difficult to compare with traditional ANOVA
    - Still under development
    - See http://afni.nimh.nih.gov/sscc/gangc/lme.html for more information

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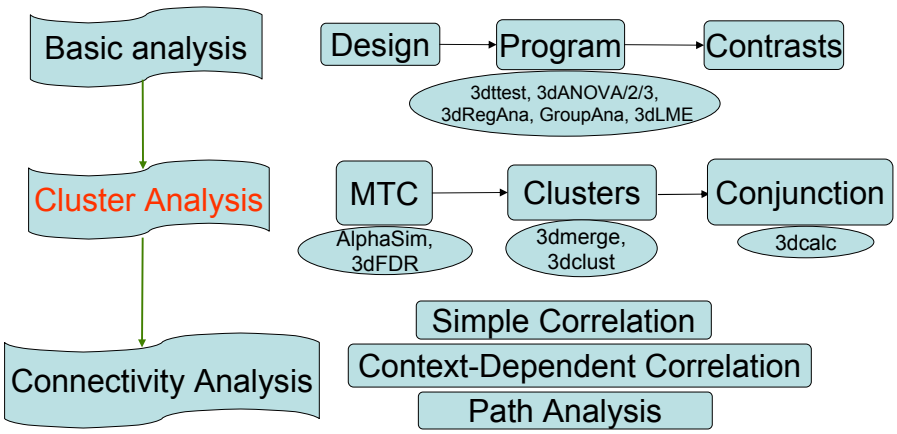
Group Analysis



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- **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 (reject H_0|when H_0 is true) = false positive = p value

      Type II = P (accept H_0|when H_1 is true) = false negative = β

      power = 1-β = probability of detecting true activation
    - Strategy: controlling type I error while increasing power (decreasing type II)
    - Significance level α (magic number 0.05) : $p < \alpha$

Justice System: Trial

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

Statistics: Hypothesis Test

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 =?

        $10000X(2)^5 \sim 300$
    - Multiple testing problem: voxel-wise statistical analysis
      - With n voxels, what is the chance to mistake ≥ 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 concerned

- **Cluster Analysis: Multiple testing correction**
  - Approaches
    - Control FWE
      - Overall significance: $\alpha_{FW} = P$ (≥ one false positive voxel in the whole brain)
      - Bonferroni correction: $\alpha_{FW} = 1-(1-p)^n \sim np$, if $p << 1/n$
        - * Use $p=\alpha/n$ as individual voxel significance level to achieve $\alpha_{FW} = \alpha$
        - * Too stringent and overly conservative: $p=10^{-8}\sim10^{-6}$
      - 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

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- **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
        - * 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

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- **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
```

Program
Restrict correcting region: ROI
Spatial correlation
Connectivity: how clusters are defined
Uncorrected *p*
Number of simulations

    - Output: 5 columns
      * Focus on the 1st and last columns, and ignore others
      * 1st column: minimum cluster size in voxels
      * Last column: alpha (α), 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↑↔ cluster size↑
    - See detailed steps at http://afni.nimh.nih.gov/sscc/gangc/mcc.html

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- **Cluster Analysis: 3dFDR**

	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	

  - 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
  - Algorithm
    - statistic (*t*) ➔ *p* value ➔ FDR (*q* value) ➔ *z* score
  - Example

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

One statistic
ROI
Arbitrary distribution of *p*
Output

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- **Cluster Analysis**: FWE or FDR?
  - FWE or FDR? Correct type I error in different sense
    - FWE: $\alpha_{FW} = P$ (≥ one false positive voxel in the whole brain)
      - Frequentist's perspective: Probability among many hypothetical activation brains
      - Used usually for parametric testing
    - FDR = expected % false + voxels among all detected voxels
      - Focus: controlling false + among detected voxels in one brain
      - More frequently used in non-parametric testing
    - 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: corrected $p = 0.05$ → 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

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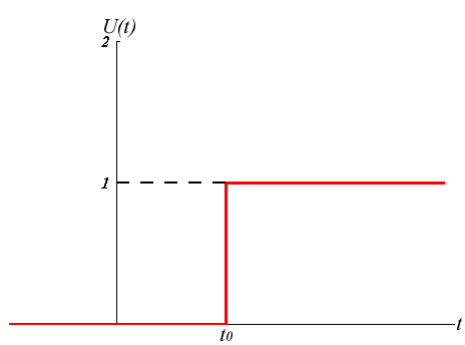
- **Cluster Analysis**: Conjunction analysis
  - Conjunction analysis
    - Common activation area: intersection
    - Exclusive activations
    - With n entities, we have 2^n possibilities (review your combinatorics!)
  - Tool: `3dcalc`
    - Heaviside unit (step function)
      defines a *On/Off* event

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



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- **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)

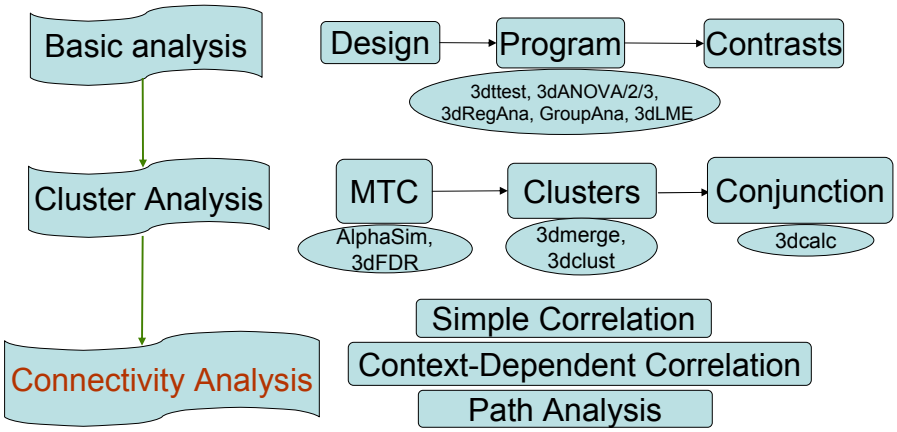
```
3dcalc -a ContrA+tlrc -b ContrB+tlrc -c ContrC+tlrc \
-expr '1*step(a-4.2)+2*step(b-4.2)+4*step(c-4.2)'   \
-prefix ConjAna
```

    - 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



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- **Connectivity: Correlation Analysis**
  - Correlation analysis (a.k.a. functional connectivity)
    - Similarity between a seed region and the rest of the brain
    - Says nothing 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

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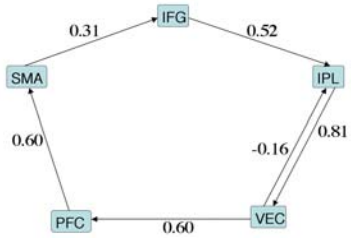
- **Connectivity: Path Analysis or SEM**
  - Causal modeling (a.k.a. structural connectivity)
    - Start with a network of ROI's
    - Path analysis
      - Assess the network based on correlations (covariances) of ROI's
      - Minimize discrepancies between correlations based on data and estimated 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 (covariance) 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

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- **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 (can be empty)
      - Some paths can be excluded
      - 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 (covariance) vs. original time series
    - Group analysis vs. individual + group

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