FMRI Connectivity Analysis in AFNI

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Structure of this lecture

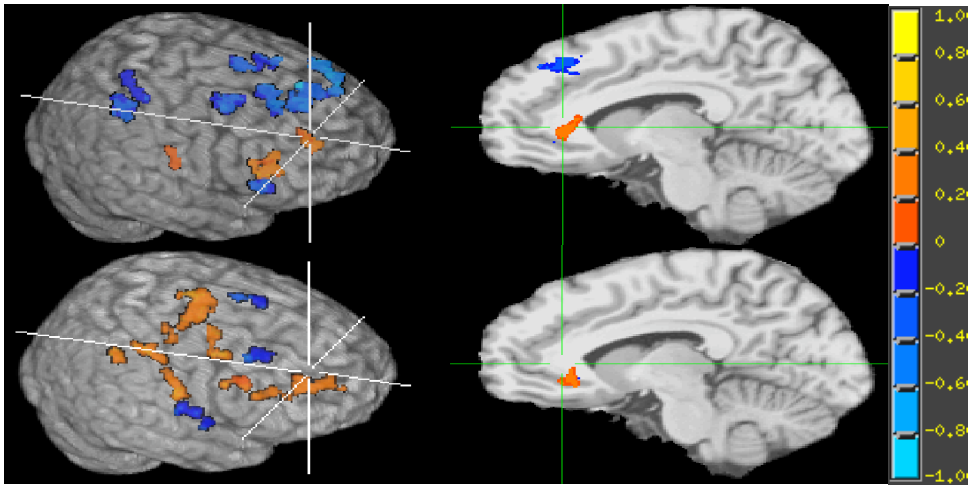
- Overview
- Correlation analysis
  - Simple correlation
  - Context-dependent correlation (PPI)
- Structural equation modeling (SEM)
  - Model validation
  - Model search
- Granger causality (GC)
  - Bivariate: exploratory - ROI search
  - Multivariate: validating – path strength among pre-selected ROIs

Overview: FMRI connectivity analysis

- All about FMRI
  - Not for DTI
  - Some methodologies may work for MEG, EEG-ERP
- Information we have
  - Anatomical structures
    - Seed-based: A seed region in a network, or
    - Network-based: A network with all relevant regions known
  - Brain output (BOLD signal): regional time series
- What can we say about inter-regional communications?
  - Inverse problem: make inference about intra-cerebral neural processes from extra-cerebral/vascular signal
  - Based on response similarity (and sequence)

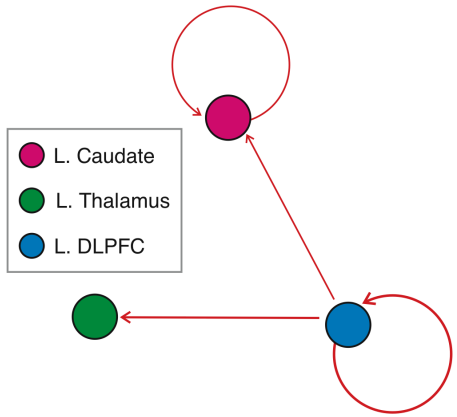
Approach I: seed-based; ROI search

- Regions involved in a network are unknown
  - Bi-regional (seed vs. whole brain) (**`3d*`**): brain volume as input
  - Mainly for ROI search
  - Popular name: functional connectivity
  - Basic, coarse, exploratory with weak assumptions
  - Methodologies: simple correlation, PPI, bivariate GC
  - Weak interpretation: may or may not indicate directionality/causality



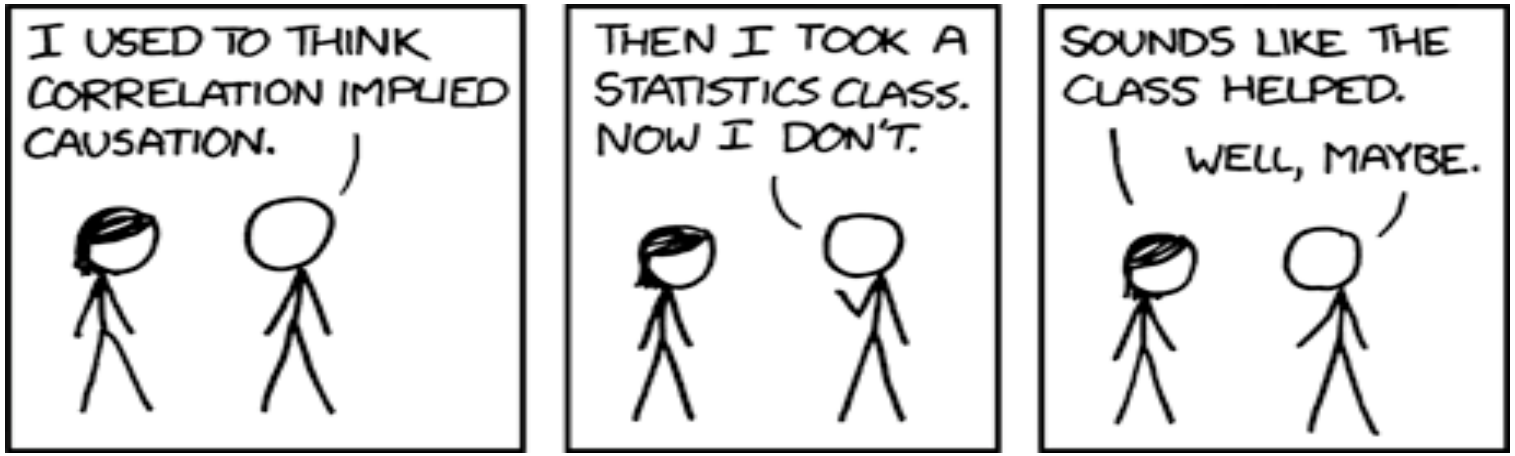
Approach II: network-based

- Regions in a network are known
  - Multi-regional (**`1d*`**): ROI data as input
  - Model validation, connectivity strength testing
  - Popular name: effective or structural connectivity
  - Strong assumptions: specific, but with high risk
  - Methodologies: SEM, multivariate GC, DCM
  - Directionality, causality (?)



Interpretation Trap: Correlation vs. Causation!

- Some analyses require fine time resolution we usually lack
- Path from (or correlation btw) A to (and) B doesn't necessarily mean causation
  - Bi-regional approach simply ignores the possibility of other regions involved
  - Analysis invalid if a relevant region is missing in a multi-regional model
- Robust: connectivity analysis < regression analysis
- Determinism in academics and in life
  - Linguistic determinism: Sapir-Whorf hypothesis



(Adopted from http://xkcd.com/552/)

Preparatory Steps

- Warp brain to standard space
  - **`adwarp, @auto-tlrc, align_epi_anat.py`**
- Create ROI
  - Sphere around a peak activation voxel: **`3dUndump –master … –srad …`**
  - Activation cluster-based (biased unless from independent data?): localizer
  - Anatomical database
  - Manual drawing
- Extract ROI time series
  - Average over ROI: **`3dmaskave –mask`**, or **`3dROIstats –mask`**
  - Principal component among voxels within ROI: **`3dmaskdump`**, then **`1dsvd`**
  - Seed voxel with peak activation: **`3dmaskdump -noijk -dbox`**
- Remove effects of no interest
  - **`3dSynthesize`** and **`3dcalc`**
  - **`3dDetrend –polort`**
  - **`RETROICORR/RetroTS.m`**
  - **`3dBandpass`**

Simple Correlation Analysis

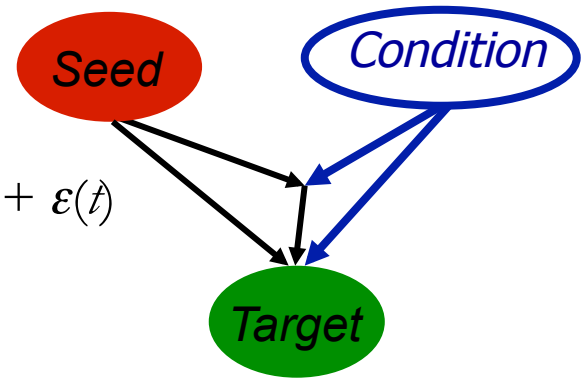
- Seed vs. rest of brain
- ROI search based on response similarity
  - Looking for regions with similar signal to seed
- **Correlation** at individual subject level
  - Usually have to control for effects of no interest: drift, head motion, physiological variables, censored time points, tasks of no interest, etc.
- Applying to experiment types
  - Straightforward for resting state experiment: default mode network (DMN)
  - With tasks: correlation under specific condition(s) or resting state?
- Program: **`3dfim+`** or **`3dDeconvolve`**
  - r: not general, but **linear**, relation; slope for standardized Y and X
  - β: slope, amount of **linear** change in Y when X increases by 1 unit
- Website: http://afni.nimh.nih.gov/sscc/gangc/SimCorrAna.html
- Interactive tools in AFNI and SUMA: **`InstaCor`**, **`GroupInstaCor`**

Simple Correlation Analysis

- Group analysis
  - Run Fisher-transformation of *r* to *Z*-score and *t*-test: **`3dttest`**
  - Take β and run *t*-test (pseudo random-effects analysis): **`3dttest`**
  - Take β + *t*-statistic and run random-effects model: **`3dMEMA`**
- **Caution**: don't over-interpret
  - Not proof for anatomical connectivity
  - No golden standard procedure and so many versions in analysis: seed region selection, covariates, r (Z)/β, bandpass filtering, …
  - Information limited if other regions present in network
  - Be careful with group comparison (normal vs. disease): assuming within-group homogeneity, can we claim
    - No between-group difference ➔ same correlation/connectivity across groups?
    - Between-group difference ➔ different correlation/connectivity across groups?

Context-Dependent Correlation

- Popularized name: Psycho-Physiological Interaction (PPI)
- 3 explanatory variables
  - Condition (or contrast) effect: $C(t)$
  - Seed effect on rest of brain: $S(t)$
  - Interaction between seed and condition (or contrast): $I(C(t), S(t))$
    - **Directionality** here!
- Model for each subject
  - Original GLM: $y = [C(t)\ \text{Others}]\ \beta + \varepsilon(t)$
  - New model: $y = [C(t)\ S(t)\ I(C(t), S(t))\ \text{Others}]\ \beta + \varepsilon(t)$
  - 2 more regressors than original model
  - Others NOT included in SPM
  - What we care for: r or β for $I(C(t), S(t))$



Context-Dependent Correlation

- How to formulate $I(C(t), S(t))$?
  - Interaction occurs at neuronal, not BOLD (an indirect measure) level
  - **Deconvolution**: derive "neuronal response" at seed based on BOLD response
    - **`3dTfitter:`** Impulse ⊗ Neuronal events = BOLD response
  - A difficult and an inaccurate process!
  - Deconvolution matters more for event-related than block experiments
  - Useful tool: **`timing_tool.py`** can convert stimulus timing into 0s and 1s
- If stimuli were presented in a resolution finer than TR
  - Use **`1dUpsample n`**: interpolate time series $n \times$ finer before deconvolution **`3dTffiter`**
  - Downsample interaction regressor back to original TR with **`1dcat`** with selector '{0..$(n)}'
- Group analysis
  - Run Fisher-transformation of r to Z-score and t-test: **`3dttest`**
  - Take β and run t-test (pseudo random-effects analysis): **`3dttest`**
  - Take β and t-statistic and run random-effects model: **`3dMEMA`**
- Website: http://afni.nimh.nih.gov/sscc/gangc/CD-CorrAna.html

PPI Caution: avoid over-interpretation

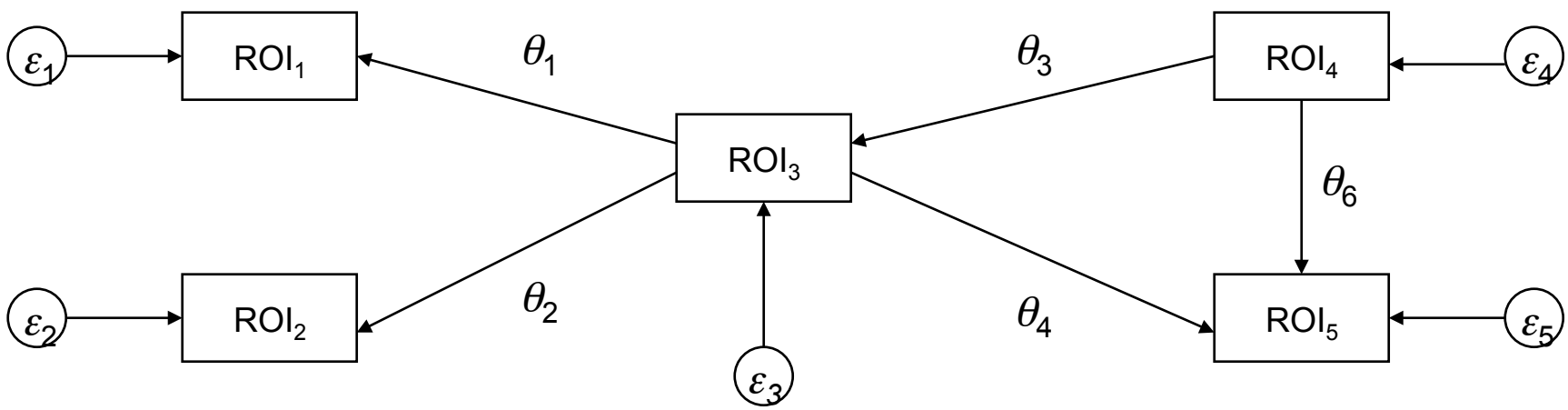
- Not proof for anatomical connectivity
- Information limited if other regions involved in the network
- Neuronal response is hard to decode: Deconvolution is very far from reliable, plus we have to assume a shape-fixed HRF (same shape regardless of condition or regions in the brain)
- Doesn't say anything about interaction between seed and target on seed
- Doesn't differentiate whether modulation is
  - Condition on neuronal connectivity from seed to target, or
  - Neuronal connectivity from seed to target on condition effect
- Be careful with group comparison (normal vs. disease group): assuming within -group homogeneity, can we claim
  - No between-group difference => same correlation/connectivity across groups?
  - Between-group difference => different correlation/connectivity across groups?

Context-Dependent Correlation: hands-on

- Data
  - Downloaded from http://www.fil.ion.ucl.ac.uk/spm/data/attention/
  - Event-related attention to visual motion experiment
  - 4 conditions: fixation, stationary, attention motion (att), no attention motion (natt)
  - TR=3.22s, 360 time points = 90 TR's/run × 4 runs, seed ROI = V2
  - All steps coded in commands.txt: tcsh –x commands.txt (~5 minutes)
- Should effects of no interest be included in PPI model?
  - Compare results between AFNI and SPM

Structural Equation Modeling (SEM) or Path Analysis

- All possible regions involved in network are included
- All regions are treated equally as endogenous (dependent) variable
- Residuals (unexplained) are exogenous (independent) variables
- Analysis based on summarized data (not original ROI times series) with model specification, covariance/correlation matrix, DF and residual error variances (?) as input



SEM: theory

- Hypothetical model $X = KX + \varepsilon$
  - X: i-th row $x_i(t)$ is i-th ROI time series
  - K: matrix of path coefficients θ's whose diagonals are all 0's
  - ε: i-th row $\varepsilon_i(t)$ is residual time series of i-th ROI
- Predicted (theoretical) covariance

  $\Sigma(\theta)=(I-K)^{-1}E[\varepsilon(t)\varepsilon(t)^T][(I-K)^{-1}]^T$ as $X = (I-K)^{-1}\varepsilon$
- ML discrepancy/cost/objective function btw predicted and estimated covariance (P: # of ROIs)

  $F(\theta) = ln\Sigma(\theta)| + tr[C\Sigma^{-1}(\theta)] - ln|C| - P$
  - Input: model specification; covariance (correlation?) matrix C; DF (calculating model fit statistic chi-square); residual error variances?
  - Usually we're interested in a network under resting state or specific condition

SEM: 1st approach - validation

- Knowing directional connectivity btw ROIs, data support model?
- Null hypothesis H_0: It's a good model
- If H_0 is **not** rejected, what are the path strengths, plus fit indices?
- Analysis for whole network, path strength estimates by-product
- 2 programs
  - **`1dSEM`** in C
    - Residual error variances as input (DF was a big concern due to limited number of time points)
    - Group level only; no CI and *p* value for path strength
    - Based on Bullmore *et al.*, How Good is Good Enough in Path Analysis of fMRI Data? NeuroImage 11, 289-301 (2000)
  - **`1dSEMr.R`** in R
    - Residual error variances not used as input
    - CI and *p* value for path strength
    - Individual and group level

SEM: 2nd approach - search

- All possible ROIs known with some or all paths are uncertain
- Estimate unknown path strengths
- Start with a minimum model (can be empty)
- Grow (add) one path at a time that lowers cost
- How to add a path?
  - Tree growth: branching out from previous generation
  - Forest growth: whatever lowers the cost – no inheritance
- Program **`1dSEM`**: only at group level
- Various fit indices other than cost and chi-square:
  - AIC (Akaike's information criterion)
  - RMSEA (root mean square error of approximation)
  - CFI (comparative fit index)
  - GFI (goodness fit index)

SEM: caution I

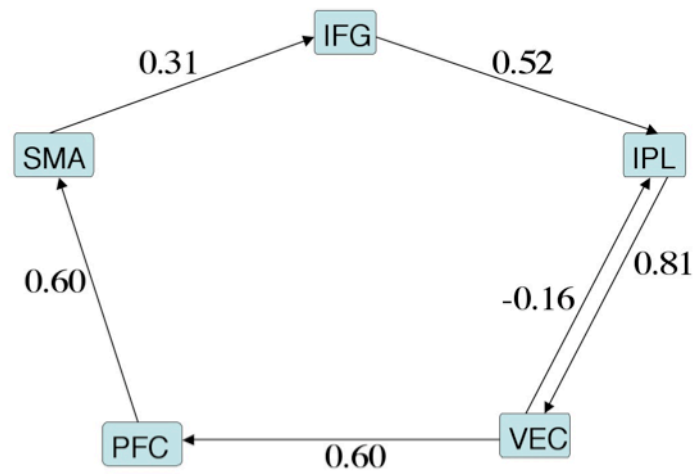
- Correlation or covariance: What's the big deal?
  - Almost **ALL** publications in FMRI use correlation as input
  - A path connecting from region A to B with strength θ
    - Not correlation coefficient
    - If A increases by one SD from its mean, B would be expected to increase by θ units (or decrease if θ is negative) of its own SD from its own mean while holding all other relevant regional connections constant
    - With correlation as input
      - May end up with different connection and/or path sign
      - Results are not interpretable
      - Difficult to compare path strength across models/groups/studies,...
  - Scale ROI time series to 1 (instead of 100 as usual)
- ROI selection very important
  - If one ROI is left out, whole analysis (and interpretation) would be invalid

SEM: caution II

- Validation
  - It's validation, not proof, when not rejecting null hypothesis
  - Different network might be equally valid, or even with lower cost: model comparison possible if nested
- Search: How much faith can we put into final 'optimal' model?
  - Model comparison only meaningful when nested (tree > forest?)
  - Is cost everything considering noisy FMRI data? (forest > tree?)
  - Fundamentally SEM is about validation, not discovery
- Only model regional relationship at current moment
  - $X = KX + \varepsilon$
  - No time delays

SEM: hands-on

- Model validation
  - Data: Bullmore *et al.* (2000)
  - Correlation as input
  - Residual error variances as input
  - **`SEMscript.csh`** maybe useful
  - **`ldSEM`**: tcsh –x commands.txt
  - **`ldSEMr.R`**: sequential mode



- Model search
  - Data courtesy: Ruben Alvarez (MAP/NIMH/NIH)
  - 6 ROIs: PHC, HIP, AMG, OFC, SAC, INS
  - Tree growth
  - Covariance as input for **`ldSEM`**
  - Shell script **`SEMscript.csh`** taking subject ROI time series and minimum model as input: tcsh –x commands.txt (~10 minutes)

Granger Causality: introduction

- Classical univariate autoregressive model AR(p)
  - $y(t) = \alpha_0 + \alpha_1 y(t\text{-}1) + \ldots + \alpha_p y(t\text{-}p) + \varepsilon(t) = \alpha_0 + \sum_{k=1}^{p} \alpha_k y(t-k) + \varepsilon(t)$; $\varepsilon(t)$ white
  - Current state depends linearly on immediate past ones with a random error
  - Why called autoregressive?
    - Special multiple regression model (on past p values)
    - Dependent and independent variable are the same
  - AR(1): $y(t) = \alpha_0 + \alpha_1 y(t\text{-}1) + \varepsilon(t)$
- What we typically deal with in GLM
  - $y = X\beta + \varepsilon$, $\varepsilon \sim N(0, \sigma^2 V)$, σ^2 varies spatially (across voxels)
  - Difficulty: V has some structure (*e.g.*, ARMA(1,1) in **`3dREMLfit`**) and may vary spatially
  - We handle autocorrelation structure in noise ε
  - Sometimes called time series regression

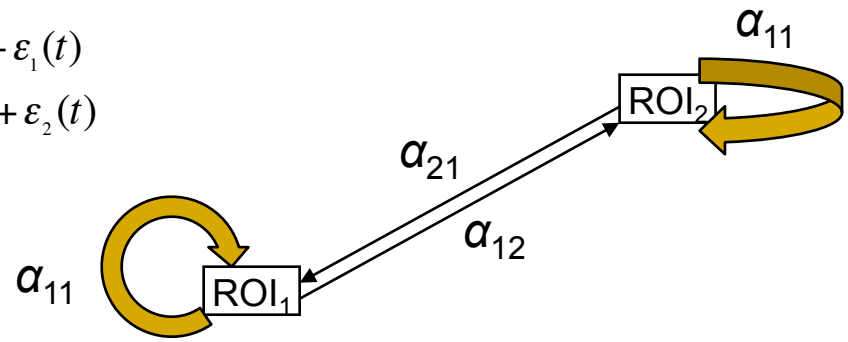
Rationale for Causality in FMRI

- Networks in brain should leave some signature (*e.g*, latency) in fine texture of BOLD signal because of dynamic interaction among ROIs
- Response to stimuli does not occur simultaneously across brain: latency
- Reverse engineering: signature may reveal network structure
- Problem: latency might be due to neurovascular differences!

Start simple: bivariate AR model

- Granger causality: A Granger causes B if
  - the time series at A provides statistically significant information about the time series at B at some time delays (order)
- 2 ROI time series, $y_1(t)$ and $y_2(t)$, with a VAR(1) model

$$y_1(t) = \alpha_{10} + \alpha_{11} y_1(t-1) + \alpha_{12} y_2(t-1) + \varepsilon_1(t)$$
$$y_2(t) = \alpha_{20} + \alpha_{21} y_1(t-1) + \alpha_{21} y_2(t-1) + \varepsilon_2(t)$$



- Assumptions
  - Linearity
  - Stationarity/invariance: mean, variance, and autocovariance
  - White noise, positive definite contemporaneous covariance matrix, and no serial correlation in individual residual time series
- Matrix form: $Y(t) = \alpha + AY(t\text{-}1) + \varepsilon(t)$, where

$$Y(t) = \begin{bmatrix} y_1(t) \\ y_2(t) \end{bmatrix} \qquad \alpha = \begin{bmatrix} \alpha_{10} \\ \alpha_{20} \end{bmatrix} \qquad A = \begin{bmatrix} \alpha_{11} & \alpha_{12} \\ \alpha_{21} & \alpha_{22} \end{bmatrix} \qquad \varepsilon(t) = \begin{bmatrix} \varepsilon_1(t) \\ \varepsilon_2(t) \end{bmatrix}$$

Multivariate AR model

- n ROI time series, $y_1(t),\ldots,y_n(t)$, with VAR(p) model

$$y_1(t) = \alpha_{10} + \sum_{k=1}^{p} \alpha_{11k} y_1(t-k) + \ldots + \sum_{k=1}^{p} \alpha_{1nk} y_n(t-k) + \varepsilon_1(t)$$

$$\vdots$$

$$y_n(t) = \alpha_{20} + \sum_{k=1}^{p} \alpha_{n1k} y_1(t-k) + \ldots + \sum_{k=1}^{p} \alpha_{nnk} y_n(t-k) + \varepsilon_n(t)$$

- Hide ROIs: $Y(t) = \alpha + A_1 Y(t\text{-}1) + \ldots + A_p Y(t\text{-}p) + \varepsilon(t)$,

$$Y(t) = \alpha + \sum_{i=1}^{p} A_i Y(t-i) + \varepsilon(t) \quad \alpha = \begin{bmatrix} \alpha_{10} \\ \vdots \\ \alpha_{n0} \end{bmatrix} \quad Y(t) = \begin{bmatrix} y_1(t) \\ \vdots \\ y_n(t) \end{bmatrix} \quad A_i = \begin{bmatrix} \alpha_{11i} & \cdots & \alpha_{1ni} \\ \vdots & \ddots & \vdots \\ \alpha_{n1i} & \cdots & \alpha_{n1i} \end{bmatrix} \quad \varepsilon(t) = \begin{bmatrix} \varepsilon_1(t) \\ \vdots \\ \varepsilon_n(t) \end{bmatrix}$$

VAR: convenient forms

- Matrix form (hide ROIs) $Y(t)=\alpha+A_1Y(t\text{-}1)+\ldots+A_pY(t\text{-}p)+\varepsilon(t)$
- Nice VAR(1) form (hide ROIs and lags): $Z(t)=\nu+BZ(t\text{-}1)+u(t)$

$$Z(t)=\begin{bmatrix} Y(t) \\ Y(t-1) \\ \vdots \\ Y(t-p+1) \end{bmatrix} \quad \nu=\begin{bmatrix} \alpha \\ 0 \\ \vdots \\ 0 \end{bmatrix} \quad B=\begin{bmatrix} A_1 & \cdots & A_{p-1} & A_p \\ I_n & \cdots & 0 & 0 \\ \vdots & \ddots & \vdots & \vdots \\ 0 & \cdots & I_n & 0 \end{bmatrix} \quad u(t)=\begin{bmatrix} \varepsilon(t) \\ 0 \\ \vdots \\ 0 \end{bmatrix}$$

- Even neater form (hide ROIs, lags and time): $Y=BZ+U$

$$Y=[Y(p+1),\cdots,Y(T)], \qquad B=[\alpha,A_1,\cdots,A_p], \qquad U=[\varepsilon(p+1),\cdots,\varepsilon(T)],$$

$$Z=\begin{bmatrix} 1 & 1 & \cdots & 1 \\ Y(p) & Y(p+1) & \cdots & Y(T-1) \\ \vdots & \vdots & \vdots & \vdots \\ Y(1) & Y(2) & \cdots & Y(T-p) \end{bmatrix}$$

  - Solve it with OLS:

$$\hat{B}=YZ^{+}=YZ^{t}(ZZ^{t})^{-1}$$

VAR extended with covariates

- Standard VAR(p) $Y(t) = \boldsymbol{\alpha} + A_1 Y(t\text{-}1) + \ldots + A_p Y(t\text{-}p) + \boldsymbol{\varepsilon}(t)$
- Covariates are all over the place!
  - Trend, tasks/conditions of no interest, head motion, time breaks (due to multiple runs), censored time points, physiological noises, etc.
- Extended VAR(p)

  $Y(t) = \boldsymbol{\alpha} + A_1 Y(t\text{-}1) + \ldots + A_p Y(t\text{-}p) + B Z_1(t) + \ldots + B_q Z_q(t) + \boldsymbol{\varepsilon}(t)$,

  where $Z_1, \ldots, Z_q$ are covariates
  - Endogenous (dependent: ROI time series)
  - Exogenous (independent: covariates) variables
  - Path strength significance: t-statistic (F in BrainVoyager)

Model quality check

- Order selection: 4 criteria (1st two tend to overestimate)
  - AIC: Akaike Information Criterion
  - FPE: Final Prediction Error
  - HQ: Hannan-Quinn
  - SC: Schwartz Criterion
- Stationarity: VAR(p) $Y(t) = \alpha + A_1 Y(t\text{-}1) + \ldots + A_p Y(t\text{-}p) + \varepsilon(t)$
  - Check characteristic polynomial $\det(I_n - A_1 z - \ldots - A_p z^p) \neq 0$ for $|z| \leq 1$
- Residuals normality test
  - Gaussian process: Jarque-Bera test (dependent on variable order)
  - Skewness (symmetric or tilted?)
  - Kurtosis (leptokurtic or spread-out?)

Model quality check (continued)

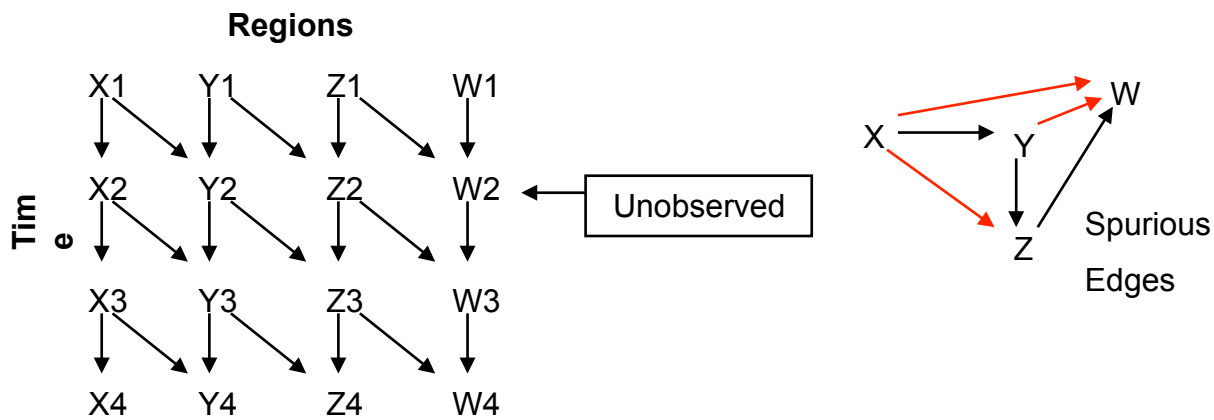
- Residual autocorrelation
  - Portmanteau test (asymptotic and adjusted)
  - Breusch-Godfrey LM test
  - Edgerton-Shukur *F* test
- Autoregressive conditional heteroskedasticity (ARCH)
  - Time-varying volatility
- Structural stability/stationarity detection
  - Is there any structural change in the data?
  - Based on residuals or path coefficients

GC applied to FMRI

- Resting state
  - Ideal situation: no cut and paste involved
  - Physiological data maybe essential?
- Block experiments
  - Duration ≥ 5 seconds?
  - Extraction via cut and paste
    - Important especially when handling confounding effects
    - Tricky: where to cut especially when blocks not well-separated?
- Event-related design
  - With rapid event-related, might not need to cut and paste (at least impractical)
  - Other tasks/conditions as confounding effects

GC: caveats

- Assumptions (stationarity, linearity, Gaussian residuals, no serial correlations in residuals, etc.)
- Accurate ROI selection
- Sensitive to lags
- Interpretation of path coefficient: slope, like classical regression
- Confounding latency due to vascular effects
- No transitive relationship: If $Y_3(t)$ Granger causes $Y_2(t)$, and $Y_2(t)$ Granger causes $Y_1(t)$, it does not necessarily follow that $Y_3(t)$ Granger causes $Y_1(t)$.
- Time resolution? Not so serious a problem? Not neuronal signal, but blurred through IRF

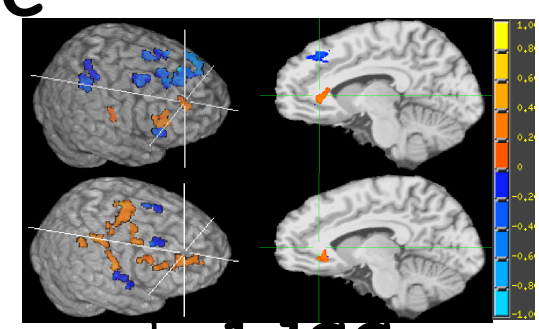


GC in AFNI

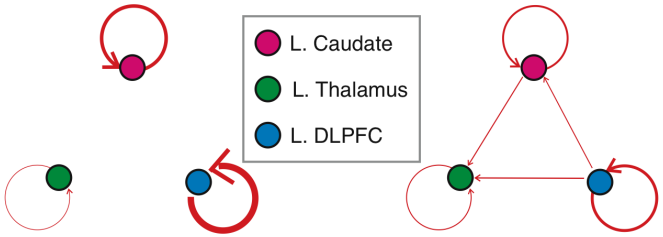
- Exploratory: ROI searching with **`3dGC`**
  - Seed vs. rest of brain
  - Bivariate model
  - 3 paths: seed to target, target to seed, and self-effect
  - Group analysis with **`3dMEMA`** or **`3dttest`**
- Path strength significance testing in network: **`1dGC`**
  - Pre-selected ROIs
  - Multivariate model
  - Multiple comparisons issue
  - Group analysis
    - path coefficients only
    - path coefficients + standard error
    - *F*-statistic (BrainVoyager)

GC: hands-on

- Exploratory: ROI searching with **`3dGC`**
  - Seed: sACC
  - Sequential and batch mode (~5 minutes)
  - Data courtesy: Paul Hamilton (Stanford)



- Path strength significance testing in network: **`1dGC`**
  - Data courtesy: Paul Hamilton (Stanford)
  - Individual subject
    - 3 pre-selected ROIs: left caudate, left thalamus, left DLPFC
    - 8 covariates: 6 head motion parameters, 2 physiological datasets
  - Group analysis
    - path coefficients only
    - path coefficients + standard errors

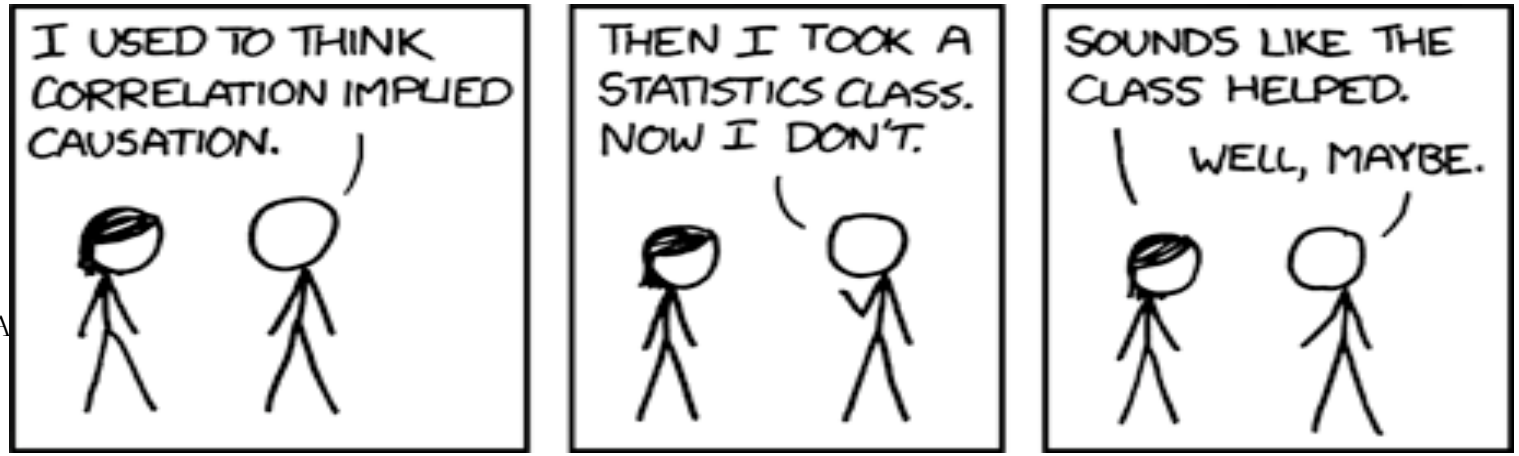


Summary: connectivity analysis

- 2 basic categories
  - Seed-based method for ROI searching
  - Network-based for network validation
- 3 approaches
  - Correlation analysis
  - Structural equal modeling
  - Granger causality
- A lot of interpretation traps
  - Over-interpretation seems everywhere
  - I may have sounded too negative about connectivity analysis
- Causality regarding the class: Has it helped you somehow?
  - Well, maybe?

Interpretation Trap: Correlation vs. Causation!

- Some analyses require fine time resolution we usually lack
- Path from (or correlation btw) A to (and) B doesn't necessarily mean causation
  - Bi-regional approach simply ignores the possibility of other regions involved
  - Analysis invalid if a relevant region is missing in a multi-regional model
- Robust: connectivity analysis < regression analysis
- Determinism in academics and in life
  - Linguistic determinism: Sapir-Whorf hypothesis



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Other approaches

- Multivariate (data-driven)
  - Techniques from machine learning, pattern recognition
  - Training + prediction
  - PCA/ICA
  - SVM: 3dsvm, plug-in
  - Kernel methods