
Connectivity Analysis in AFNI

File: Connectivity.pdf

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SSCC/NIMH/NIH/HHS



Why connectivity?

- Understanding communications in brain networks
 - More interesting than regional activations
 - May indicate some abnormal situations (ASD, schizophrenia)
 - Connectome!!!
- Many connectivity methods
 - People try to squeeze the data as hard as possible
 - Unlike activation detection, connectivity analysis methods are usually unsatisfactory or controversial
 - Two aspects: poor data and poor models
 - Publish or perish?
 - Only a few introduced here
 - Focus more on understanding methods than recommending

Structure of this lecture

- Two categories of connectivity analysis
 - Seed-based (vs. functional connectivity)
 - Network-based (vs. effective connectivity)
- Seed-based analysis
 - Simple correlation
 - Context-dependent correlation (PPI)
 - Seed-based bivariate autoregression (Granger)
- Network-based analysis
 - Structural equation modeling (SEM)
 - Vector autoregression (VAR) (aka Granger causality)
 - Structural vector autoregression (SVAR)

Overview: Connectivity analysis

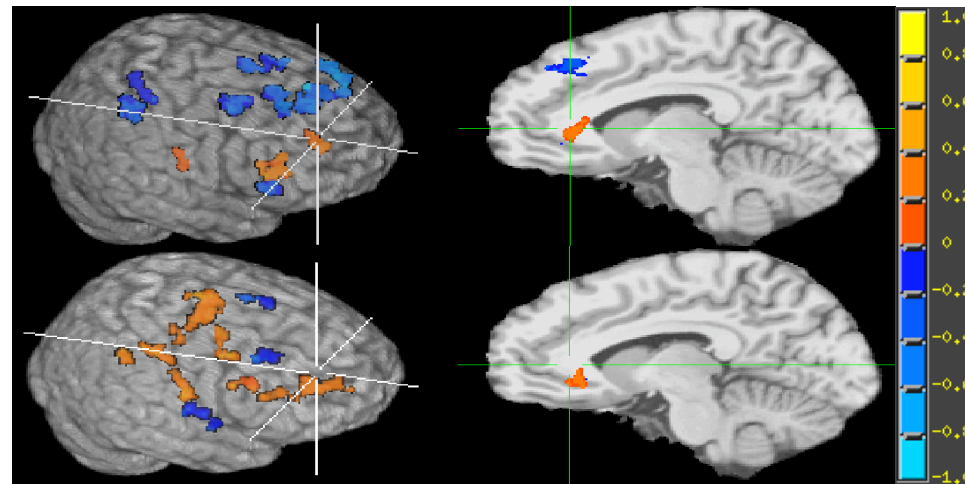
- Typical fMRI data analysis
 - Massively univariate (voxel-wise) regression: $y = X\beta + \epsilon$
 - Relatively robust and reliable
 - May infer regions involved in a task/state, but can't say much about the details of a network
- Network analysis
 - Information
 - Seed region, some or all regions in a network
 - Neuroimaging data (fMRI, MEG, EEG): regional time series
 - Inferring interregional communications
 - Inverse problem: infer neural processes from BOLD signal
 - Based on response similarity (and sequence)
 - Difficult and usually not so reliable

Overview: Connectivity analysis

- Two types of network analysis
 - Not sure about ALL the regions involved
 - **Seed-based**: use a seed region to search for other ROIs
 - If all regions in a network known
 - Prior knowledge
 - **Network-based**: A network with all relevant regions known
 - Everything is relative: No network is fully self-contained
- Currently most methods are crude
 - Models: underlying assumptions not met
 - Data quality: temporal resolution, low signal-to-noise ratio, poor understanding of fMRI signal

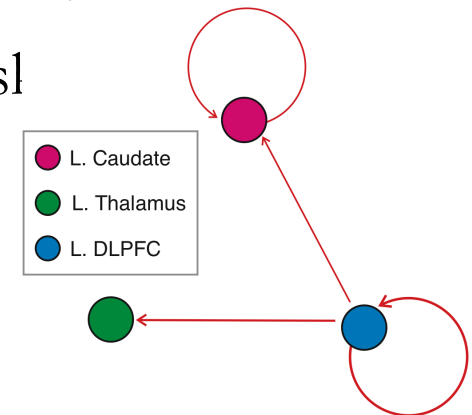
Seed-based analysis: ROI search

- 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 autoregression
 - **Weak interpretation**: may or may not indicate directionality/causality



Network-based analysis

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



Common Preparatory Steps

- Warp brain to standard space
 - **Uber_subject.py**, **uber_align_test.py**, **adwarp**, **@auto-tlrc**, **align_epi_anat.py**
- Create ROI
 - Peak voxel or sphere around a peak voxel: **3dUndump -master ... -srad ...**
 - Activation cluster-based (biased unless from independent data?)
 - Anatomical database or manual drawing
- Extract ROI time series
 - Average over ROI: **3dmaskave -quiet -mask**, or **3dROIstats -quiet -mask**
 - Principal component among voxels within ROI: **3dmaskdump**, then **1dsvd**
 - Seed voxel with peak activation: **3dmaskdump -noijk -dbox**
- Remove effects of no interest
 - **3dSynthesize** (effects of no interest) and **3dcalc** (effects of interest)
 - **3dDetrend -polort** (trend removal)
 - **RETROICOR/RetroTS.m** (physiological confounds)
 - **3dBandpass** (bandpass filtering)
 - **@ANATICOR** (resting state data)

Simple Correlation Analysis

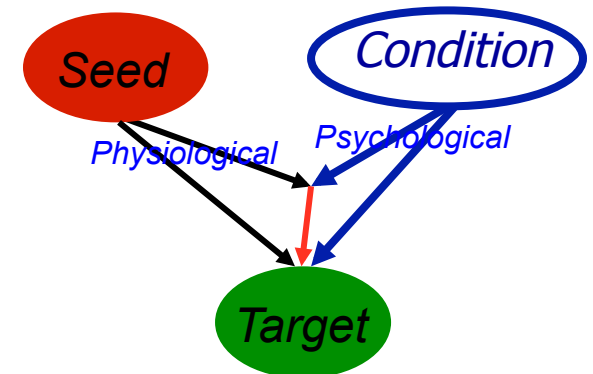
- Resting state data analysis: seed vs. rest of brain
- ROI search based on response similarity
 - Looking for regions with similar signal to seed: spontaneous fluctuations
- **Correlation** at individual subject level
 - Usually have to control for effects of no interest: drift, head motion, physiological 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 a specific condition or resting state?
- Program: **3dDeconvolve** or **afni_proc.py**
 - Original regression: $y = X \mathbb{W} + \mathbb{W}(t)$
 - New model: $y = [X \ S(t)] \mathbb{W} + \mathbb{W}(t)$
 - r : **linear** correlation; slope for standardized Y and X
 - β : slope, amount of **linear** change in Y when X increases by 1 unit

Simple Correlation Analysis

- Group analysis
 - Run Fisher-transformation of r to Z-score and t -test: **3dttest++**
- Interactive tools in AFNI and SUMA:
uber_subj.py, InstaCor, GroupInstaCor
- **Caveats:** don't over-interpret
 - Correlation: crude measurement at the presence of significant noise
 - Only linearity relationship
 - Correlation does not necessarily mean causation: no proof for anatomical connectivity (*e.g.*, more than two regions in a network)
 - **No golden standard procedure** and so many versions in analysis: seed region selection, confounds, head motions, **preprocessing steps**, ...
 - **Measurement error** problem: underestimation, attenuated bias

Context-Dependent Correlation

- Popular name: Psycho-Physiological Interaction (PPI)
- Regression analysis at individual level
 - Brain response varies in magnitude across multiple trials (repetitions)
 - Habituations, random fluctuations, ...
 - Regression only accounts for the **AVERAGE** response across trials
 - Trial-to-trial fluctuations treated as noise (residuals)
 - Do the fluctuations provide some information about the brain network?
- Image three components
 - Main effect of condition (or contrast): $C(t)$
 - Main effect of seed on target: $S(t)$
 - Interaction between the two effects: $I(C(t), S(t))$
 - **Implicit directionality assumption** here!



Context-Dependent Correlation

- Model for each subject
 - Original regression: $y(t) = [C(t) \text{ Others}] \beta + \epsilon(t)$
 - New model: $y(t) = [C(t) S(t) I(C(t), S(t)) \text{ Others}] \beta + \epsilon(t)$
 - $C(t)$ and $S(t)$: like main effects in a two-way ANOVA
 - $I(C(t), S(t))$: interaction (regressor of interest)
 - 2 more regressors than original model: $S(t)$, $I(C(t), S(t))$
 - Should effects of no interest be included in the model?
 - **Others** NOT included in SPM
 - What we care for: β for $I(C(t), S(t))$
 - $I(C(t), S(t))$ accounts for the variability in addition to $C(t)$ and $S(t)$
 - Symmetrical modulation

Context-Dependent Correlation

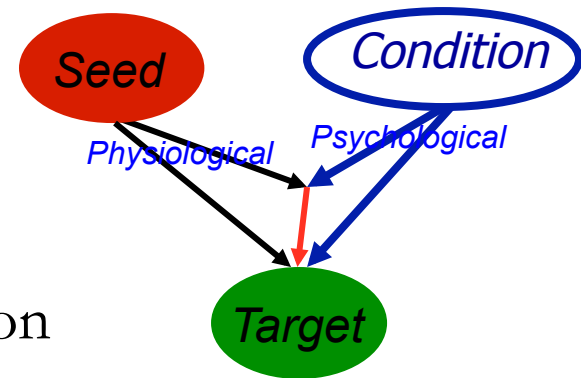
- How to formulate interaction $I(C(t), S(t))$?
 - Interaction at neuronal, not BOLD (an indirect measure), level
 - **Deconvolution**: derive neuronal response from BOLD response
 - Assuming standard (fixed) impulse response
 - **3dTfitter**: Impulse \boxtimes Neural events = BOLD response; Gamma \boxtimes $NE(t) = S(t)$
 - Deconvolution matters more for event-related than block experiments
 - Interaction at neuronal level – **3dcalc**: $NE(t) \times C(t) = NI(t)$
 - **timing_tool.py** converts stimulus timing into 0s and 1s
 - 1s and -1s for contrast, and 1s and 0s for condition vs. baseline

Context-Dependent Correlation

- How to formulate interaction $I(C(t), S(t))$?
 - Interaction at BOLD level - convolution – **waver**: $\text{Gamma} \otimes \text{NI}(t) = I(C(t), S(t))$
 - If stimuli presented in a higher resolution than TR – not TR-locked
 - Up-sample first: use **1dUpsample n** to interpolate $S(t)$ $n \otimes$ finer before deconvolution **3dTffiter**
 - Down-sample interaction $I(C(t), S(t))$ back to original TR: **1dcat** with selector '{0..\$(n)}'
 - Regression: $y(t) = [C(t) \ S(t) \ I(C(t), S(t)) \ \text{Others}] \otimes + \otimes(t) -$
3dDeconvolve
 - Website: <http://afni.nimh.nih.gov/sscc/gangc/CD-CorrAna.html>
- Group analysis: Take $\beta (+t)$: **3dttest (3dMEMA)**

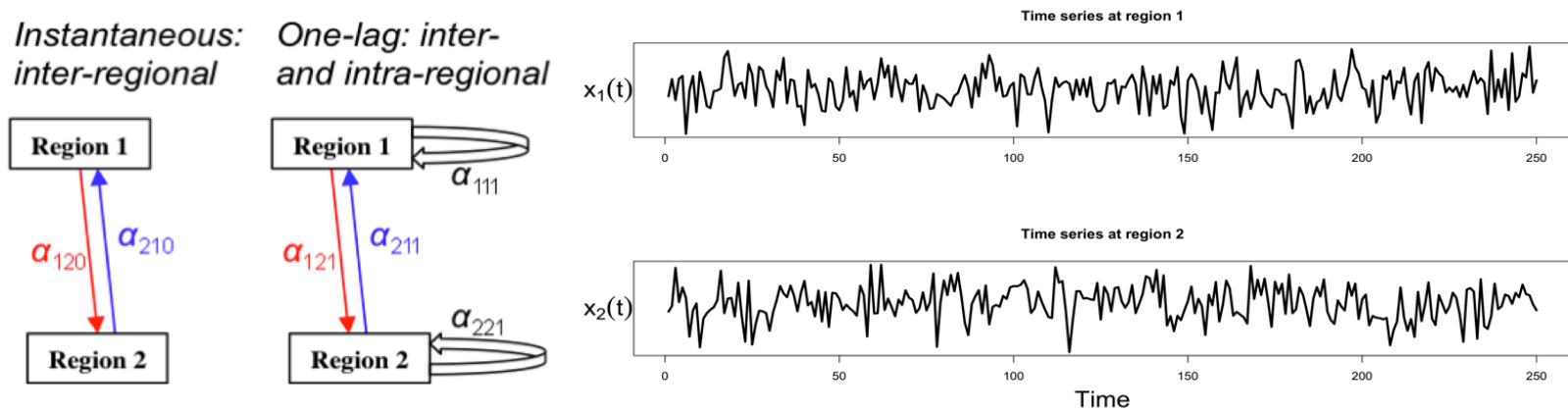
PPI Caveats

- No proof for anatomical connectivity
 - Correlation does not necessarily mean causation
 - Only modeling interactions between two regions
- Big noise: measurement error in regression
 - Poor understanding of BOLD
 - Neural response hard to decode: **Deconvolution** is not so reliable, with assumption of a fixed-shape HRF, same across trials/conditions/regions/subjects/groups
 - Noisy seed time series: attenuation or regression dilution
- Directionality presumption
- No information about interaction between condition and target on seed
- No differentiation whether modulation is
 - Condition on neuronal connectivity from seed to target, or
 - Neural connectivity from seed to target on condition effect



Network-Based Modeling: a toy example

- A network with two regions: both **contemporaneous** and **delayed**



- Within-region effects: lagged correlation
- Cross-regions effects: both instantaneous and lagged

$$x_1(t) = c_1 + \alpha_{120}x_2(t) + \alpha_{111}x_1(t-1) + \alpha_{121}x_2(t-1) + \varepsilon_1(t)$$

$$x_2(t) = c_2 + \alpha_{210}x_1(t) + \alpha_{211}x_1(t-1) + \alpha_{221}x_2(t-1) + \varepsilon_2(t)$$

- If we have time series data from the two regions
 - Can we evaluate the above model?
 - Estimate and make inferences about the connections (α values)?

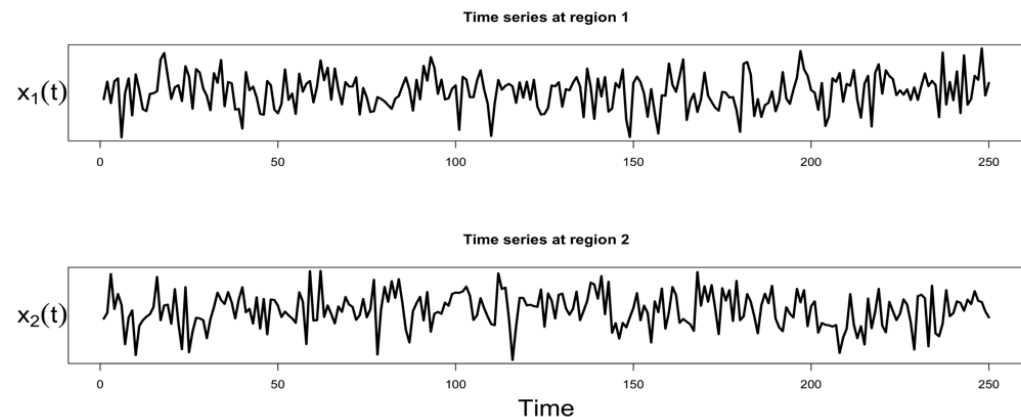
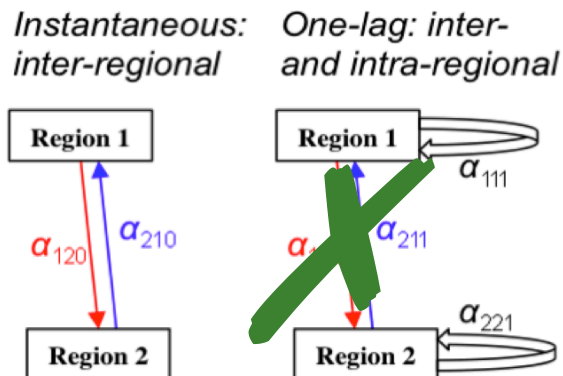
Structure Equation Modeling (SEM): a toy example

- A network with two regions: **no delayed effects**
 - **No within-region effects**: no lagged effects – **no temporal correlation!**
 - Cross-region effects: instantaneous correlation only; **no lagged effects**

$$x_1(t) = c_1 + \alpha_{120}x_2(t) + \varepsilon_1(t)$$

$$x_2(t) = c_2 + \alpha_{210}x_1(t) + \varepsilon_2(t)$$

- If we have time series data from the two regions
 - Can we evaluate the above model?
 - Estimate and make inferences about the α values?



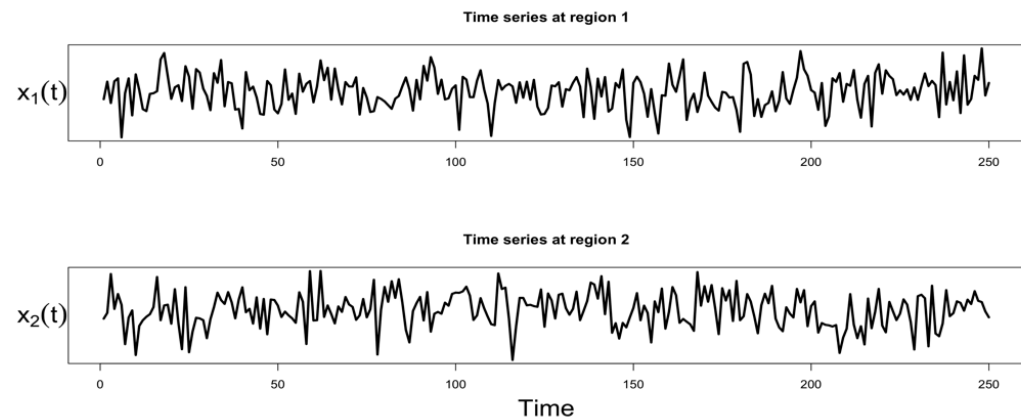
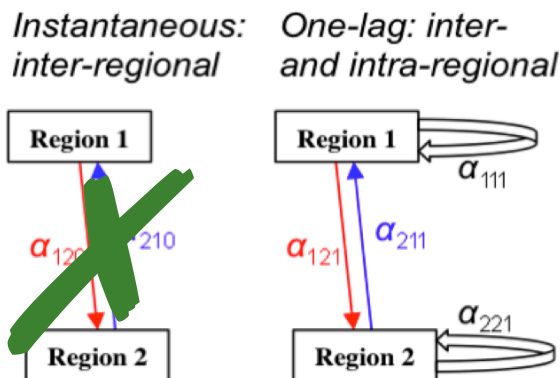
Vector Autoregressive (VAR) Modeling: a toy example

- A network with two regions: **no contemporaneous effects**
 - Within-region effects: lagged effects
 - Cross-regions effects: lagged effects only; **no instantaneous effects**

$$x_1(t) = c_1 + \alpha_{111}x_1(t-1) + \alpha_{121}x_2(t-1) + \varepsilon_1(t)$$

$$x_2(t) = c_2 + \alpha_{211}x_1(t-1) + \alpha_{221}x_2(t-1) + \varepsilon_2(t)$$

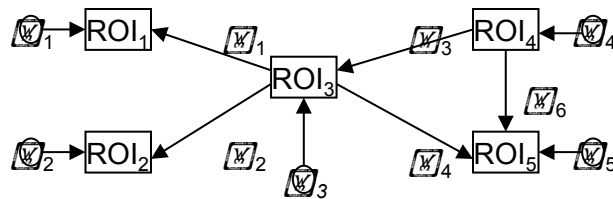
- If we have time series data from the two regions
 - Can we evaluate the above model?
 - Estimate and make inferences about the α values?



Structure Equation Modeling (SEM) or Path Analysis

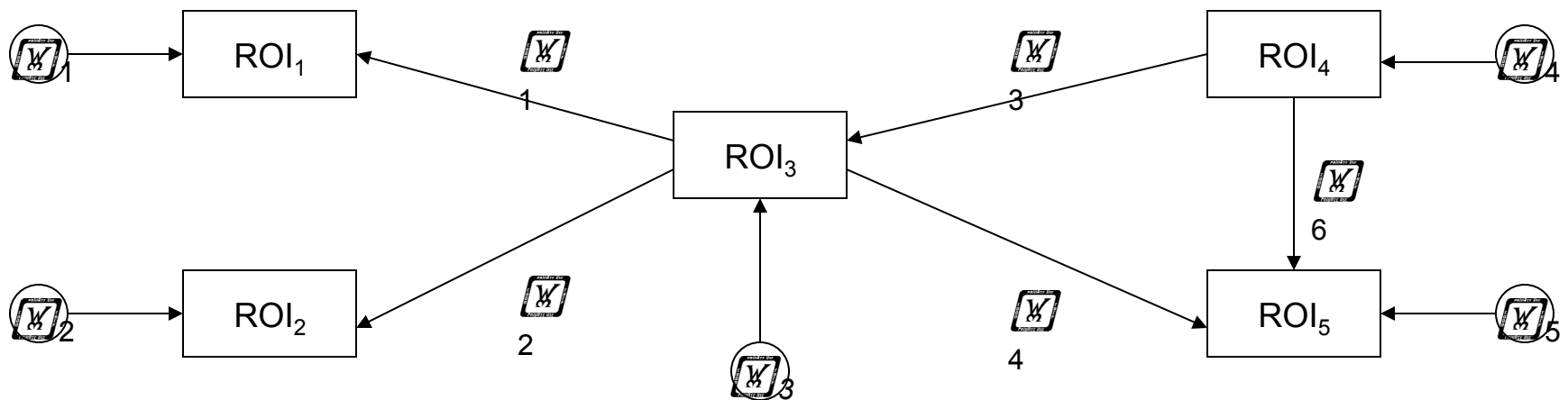
- General model for a network of n regions $X^*(t) = A_0 X^*(t) + \varepsilon(t)$
 - Only consider instantaneous effects; assumes **no delayed effects**
 - Data centered around mean; if possible, remove all confounding effects
 - Parameters in A_0 code for cross-region path strength; **zero diagonals**
 - $\varepsilon(t) \sim N(0, \Psi)$, Ψ : **diagonal matrix** (interregional correlations: A_0)
- Solving SEM: guess directional connections based on correlations
 - Compare covariance matrix from data with the one from the model

$$\Sigma = (I - A_0)^{-1} \varepsilon \varepsilon^T (I - A_0)^{-T} = (I - A_0)^{-1} \Psi (I - A_0)^{-T}$$
 - One problem: we can't solve SEM if all parameters in A_0 are unknown!
 - Totally $n(n+1)/2$ simultaneous equations; $n(n-1) + n = n^2$ unknowns!
 - Can only allow at most $n(n-1)/2$ paths, half of the off-diagonals
 - Have to fix the rest paths (at least $n(n-1)/2$) to 0 or known values



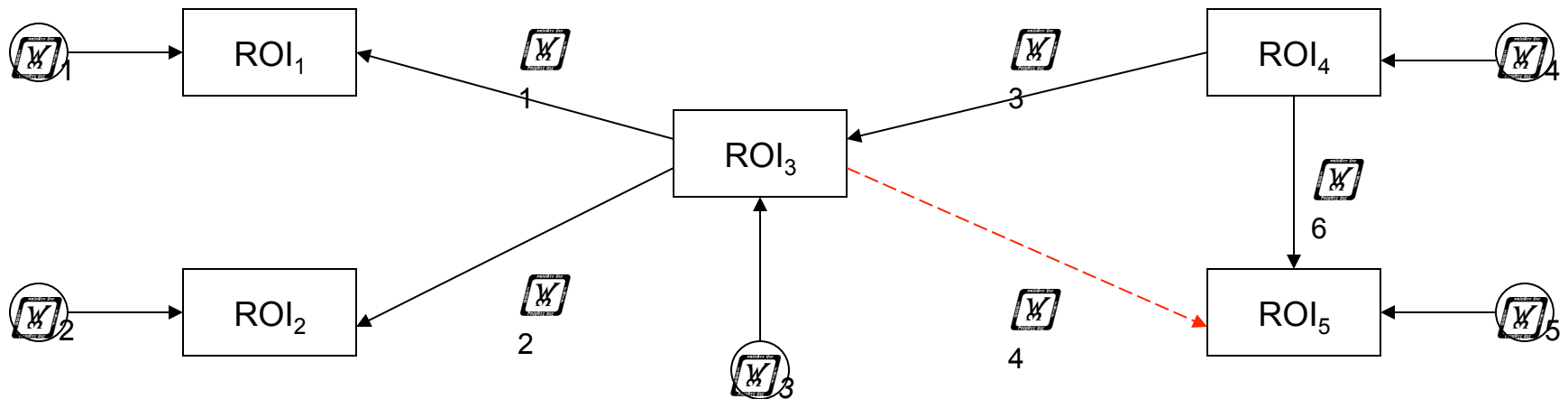
SEM: Model Validation

- Null hypothesis H_0 : It's a good model about instantaneous network
 - Knowing directional connectivity btw ROIs, does data support model?
 - Want to see model (H_0) **not** rejected
 - $\chi^2(n(n-1)/2-k)$ -test: **badness-of-fit**
 - Fit indices (AIC, CFI, GFI,): balance between optimization and model complexity
 - Input: **model specification**, **covariance/correlation matrix**, *etc.*
 - If H_0 is **not** rejected, estimate path strengths



SEM: Model Comparison and Search

- Comparing two **nested** models through $\chi^2(1)$ -test
 - For example, not sure about a path
- Search all possible models
 - Sounds appealing: often seen in literature
 - Problematic: data-driven vs. theory-based
 - **Learn from data, and don't let data be your master!**



SEM: Serious Problems

- **Most models are like bikinis!**
 - Correlations as input in SEM: popular practice
 - Usually practiced in social science studies for scaling issues
 - Save DFs in fMRI data analysis
 - Path coefficients not interpretable
 - Can't make statistical inferences: t -stat and CI, if provided, are incorrect
 - Assumption of no delayed effects
 - Within-region temporal correlations ignored
 - Cross-regions: delayed interactions ignored
 - Data preprocessing: Have to remove all confounding effects
 - Individual subjects vs. group
 - How to combine multiple multiple subjects
 - Fixed vs. random-effects analysis
-

Vector Autoregression (VAR)

- General model for a network of n regions VAR(p)
 - $X(t) = A_1X(t-1) + \dots + A_pX(t-p) + c_1z_1(t) + \dots + c_qz_q(t) + \mathcal{E}(t)$
 - Only focus on lagged effects: Current state depends linearly on history
 - Instantaneous effects modeled, but left in residuals as effects of no interest
 - Confounding (**exogenous**) effects can be incorporated as part of the model
 - Slow drift, head motion, physiological confounds, **time breaks**, conditions of no interest
 - Unlike SEM, only minimal pre-processing needed (slice timing + motion correction)
 - Parameters in A_i code for cross-region path strength: Meaning of path coefficients
 - Assumptions
 - Linearity; Stationarity/invariance: mean, variance, and auto-covariance
 - $\mathcal{E}(t) \sim N(0, \Psi)$, Ψ : **not diagonal matrix** (positive definite contemporaneous covariance); no serial correlation in individual residual time series
- Rationale for VAR(p)
 - Response to stimuli does not occur simultaneously across brain: latency
 - However, is data time resolution fine enough with TR = 2 sec???

Solving VAR

- Model $X(t) = A_1X(t-1) + \dots + A_pX(t-p) + c_1z_1(t) + \dots + c_qz_q(t) + \epsilon(t)$
 - Order selection with 4 criteria (1st two tend to overestimate)
 - AIC: Akaike Information Criterion
 - FPE: Final Prediction Error
 - HQ: Hannan-Quinn
 - SC: Schwartz Criterion
- Solve VAR with OLS
 - No need to specify connections as in SEM
 - Obtain estimates of all elements in A_i , and make statistical inferences based on t -statistic for each path
 - **Data driven** instead of model validation?
 - Model tuning when some covariates are not significant
- VAR as a seed-based analysis
 - Bivariate autogression: use seed to search for regions that may form a network with the seed
 - **3dGC** (vs. **1dGC**): should have been called 3dVAR (vs. 1dVAR)

VAR Model Quality Check

- Stationarity: VAR(p) $Y(t) = \alpha + A_1 Y(t-1) + \dots + A_p Y(t-p) + \epsilon(t)$
 - Check characteristic polynomial $\det(I_n - A_1 z - \dots - A_p z^p) \neq 0$ 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?)
- 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

VAR: Serious Problems

- Data sampling rate: time resolution
 - Cross-region interactions occur probably at ms level, but usually $TR = 2s$ in fMRI time series (TR could be 100-200 ms with single-slice scanning)
 - Will VAR(1) catch the real lagged effects across regions???



- With coarse sampling, the instantaneous effects will more likely reveal the real network than the lagged effects
- Endogeneity problem or over-fitting: data driven

Network-Based Modeling: a toy example

- A network with two regions: both **contemporaneous** and **delayed**

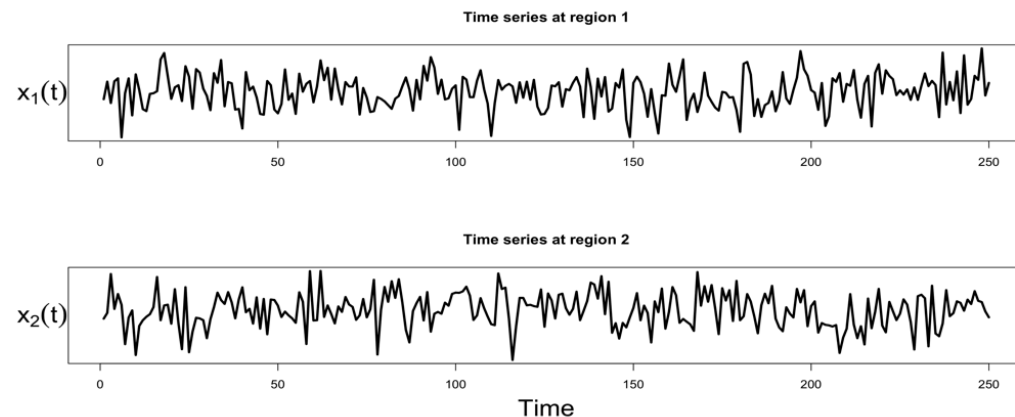
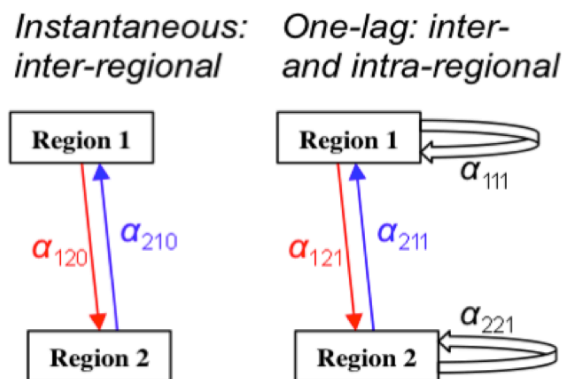
- Within-region effects: lagged correlation
- Cross-regions effects: both instantaneous and lagged

$$x_1(t) = c_1 + \alpha_{120}x_2(t) + \alpha_{111}x_1(t-1) + \alpha_{121}x_2(t-1) + \varepsilon_1(t)$$

$$x_2(t) = c_2 + \alpha_{210}x_1(t) + \alpha_{211}x_1(t-1) + \alpha_{221}x_2(t-1) + \varepsilon_2(t)$$

- If we have time series data from the two regions

- Can we evaluate the above model?
- Estimate and make inferences about the α values?



One World United Under One Flag!

- Why don't we just combine SEM and VAR?
 - No reason we shouldn't or cannot
 - Called **Structural Vector Autoregression** (SVAR)!
 - Accounts for variability from both instantaneous and lagged effects
 - Improves model quality and statistical power
 - Incorporates covariates, and involves minimum pre-processing
- General SVAR(p) model
 - $X(t) = A_0 X(t) + A_1 X(t-1) + \dots + A_p X(t-p) + c_1 z_1(t) + \dots + c_q z_q(t) + B \boldsymbol{\varepsilon}(t)$
 - A_0 represents the cross-region instantaneous effects
 - Diagonals are 0
 - A_i represents both within-region and cross-region lagged effects
 - B is a diagonal matrix so that $\boldsymbol{\varepsilon}(t) \sim N(0, I)$
 - All the cross-region instantaneous effects are contained in A_0

Solving SVAR

- $X(t) = A_0 X(t) + A_1 X(t-1) + \dots + A_p X(t-p) + \mathbf{c}_1 z_1(t) + \dots + \mathbf{c}_q z_q(t) + B \boldsymbol{\varepsilon}(t)$

- Equivalence to a reduced VAR(p) model

$$X(t) = A_1^* X(t-1) + \dots + A_p^* X(t-p) + \mathbf{c}_1^* z_1(t) + \dots + \mathbf{c}_q^* z_q(t) + \boldsymbol{\varepsilon}^*(t)$$

$$A_i^* = (I - A_0)^{-1} A_i, \quad \mathbf{c}_j^* = (I - A_0)^{-1} \mathbf{c}_j, \quad \boldsymbol{\Sigma}^*(t) = (I - A_0)^{-1} B \boldsymbol{\Sigma}(t)$$

- Solve the reduced VAR(p), obtain estimates of A_i^* , \mathbf{c}_j^* , and residual covariance $\boldsymbol{\Sigma}^*$

- Solve $(I - A_0)^{-1} B B^T (I - A_0)^{-1} = \boldsymbol{\Sigma}^*$ through ML. Similar to SEM:

- Totally $n(n+1)/2$ simultaneous equations; $n(n-1) + n = n^2$ unknowns!
- Can only allow at most $n(n-1)/2$ paths in A_0 , half of the off-diagonals
- Have to fix the rest paths (at least $n(n-1)/2$) to 0 or known values
- Model validation, comparison, and search for the instantaneous network A_0

- Finally update A_i (and \mathbf{c}_j) for the lagged effects

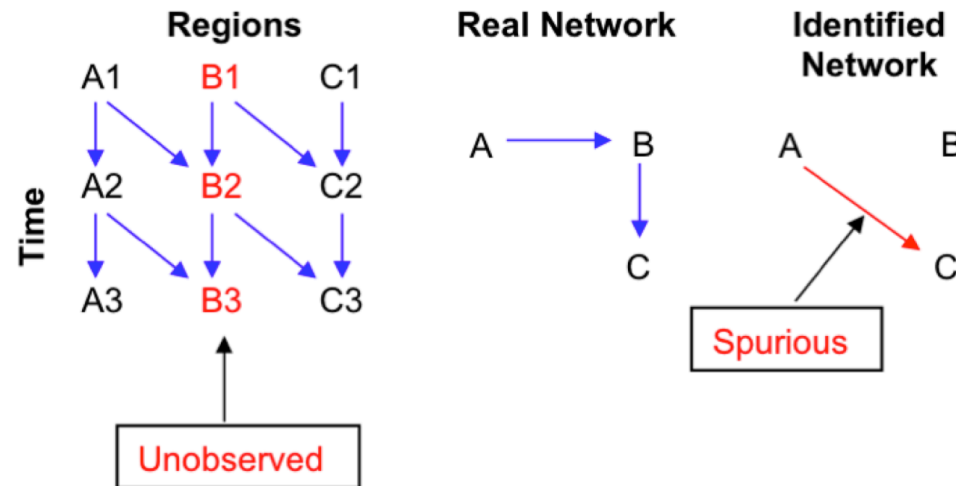
- AFNI program [1dSVAR.R](#)

What can we do with 1dSVAR

- If time resolution is too coarse (*e.g.*, fMRI): Model validation/ comparison/search of the instantaneous network while accounting for the lagged effects
 - Knowing directional connectivity btw ROIs, does data support model?
 - Want to see model (H_0) **not** rejected
 - $\chi^2(n(n-1)/2-k)$ -test: **badness-of-fit**
 - Fit indices (AIC, CFI, GFI,): balance between optimization and model complexity
 - If H_0 is **not** rejected, what are the path strengths?
- If time resolution is good (*e.g.*, MEG/EEG)
 - Both instantaneous and lagged effects are of interest?
- SEM+VAR
 - Lagged effects: data-driven; **safe but inefficient (over-fitting)**
 - Instantaneous effects: theory/hypothesis-based; **powerful but risky**
 - Various possibilities: *e.g.*, borrow DFs for instantaneous effects from lagged effects?
- Group analysis: MEMA

SVAR: caveats

- Assumptions (stationarity, linearity, Gaussian residuals, no serial correlations in residuals, etc.)
- Accurate ROI selection: If an essential region is missing



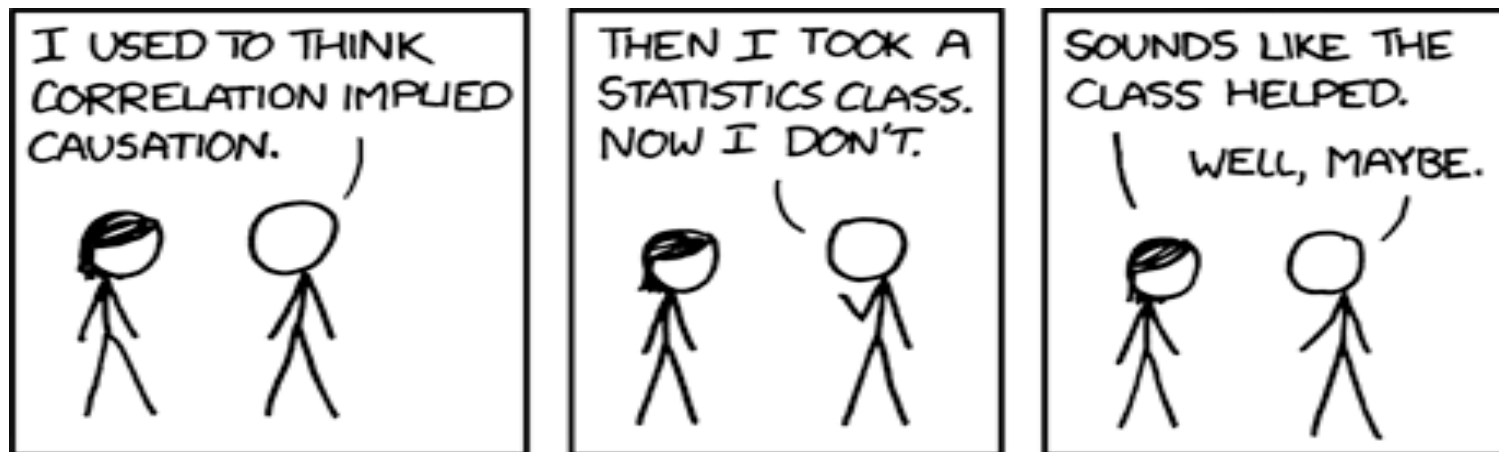
- Sensitive to lags
- Confounding latency due to HDR variability and vascular confounds
- Overfitting
- Model comparison/search
 - **Learn from data, but don't let data be your teacher!**

SVAR 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

SVAR: Why not Granger Causality

- Causality: philosophical and physiological/anatomical; effective?
- Granger causality: A Granger causes B if time series at A provides **statistically significant** information about time series at B at some time delays (order)
 - Causes must temporally precede effects
 - Causality can be inferred from an F - or χ^2 -test that shows the amount of variability of overall lagged effects each connection accounts for
- Both instantaneous and lagged effects are modeled in SVAR



Network-based Analysis 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: **1dSVAR**
 - Pre-selected ROIs
 - SVAR model
 - Multiple comparisons issue
 - Group analysis
 - path coefficients only
 - path coefficients + standard error
 - F -statistic (BrainVoyager)

Keep in mind

- Statisticians, like artists, have the bad habit of falling in love with their models. (George Box)
- If you torture the data enough, nature will always confess. (Ronald Coase)
- **Models are bikinis!**