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 $\mathbf{1d*}$: 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 $X$
  - $\beta$: slope, amount of **linear** change in $Y$ when $X$ increases by 1 unit
- Interactive tools in AFNI and SUMA: **InstaCor**, **GroupInstaCor**
Simple Correlation Analysis

- **Group analysis**
  - Run Fisher-transformation of $r$ to $Z$-score and $t$-test: \texttt{3dttest}
  - Take $\beta$ and run $t$-test (pseudo random-effects analysis): \texttt{3dttest}
  - Take $\beta + t$-statistic and run random-effects model: \texttt{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, $r (Z)/\beta$, 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 $\Rightarrow$ same correlation/connectivity across groups?
    - Between-group difference $\Rightarrow$ different correlation/connectivity across groups?
Context-Dependent Correlation

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

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/](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

\[
\begin{align*}
\epsilon_1 & \rightarrow ROI_1 \\
\epsilon_2 & \rightarrow ROI_2 \\
\epsilon_3 & \rightarrow ROI_3 \\
\epsilon_3 & \rightarrow ROI_4 \\
\epsilon_5 & \rightarrow ROI_5
\end{align*}
\]
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 \( \theta \)'s whose diagonals are all 0's
  - \( \varepsilon \): \( i \)-th row \( \varepsilon_i(t) \) is residual time series of \( i \)-th ROI

- Predicted (theoretical) covariance
  \[
  \Sigma(\theta) = (I-K)^{-1}E[\varepsilon(t)\varepsilon(t)^T][(I-K)^{-1}]^T \quad \text{as} \quad X = (I-K)^{-1}\varepsilon
  \]

- 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
  - 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)
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 $\theta$
  - Not correlation coefficient
  - If A increases by one SD from its mean, B would be expected to increase by $\theta$ units (or decrease if $\theta$ 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
  - \texttt{SEMscript.csh} maybe useful
  - \texttt{1dSEM}: tcsh –x commands.txt
  - \texttt{1dSEMr.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 \texttt{1dSEM}
  - Shell script \texttt{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)
  - \( y(t) = \alpha_0 + \alpha_1 y(t-1) + \ldots + \alpha_p y(t-p) + \epsilon(t) = \alpha_0 + \sum_{k=1}^{p} \alpha_k y(t-k) + \epsilon(t) \) 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): \( y(t) = \alpha_0 + \alpha_1 y(t-1) + \epsilon(t) \)

- What we typically deal with in GLM
  - \( y = X\beta + \epsilon, \epsilon \sim N(0, \sigma^2 V) \), \( \sigma^2 \) 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 \( \epsilon \)
  - 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
  
  \[
  y_1(t) = \alpha_{10} + \alpha_{11} y_1(t-1) + \alpha_{12} y_2(t-1) + \epsilon_1(t) \\
  y_2(t) = \alpha_{20} + \alpha_{21} y_1(t-1) + \alpha_{22} y_2(t-1) + \epsilon_2(t)
  \]

- 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: \( Y(t) = \alpha + AY(t-1) + \epsilon(t) \), where
  
  \[
  Y(t) = \begin{bmatrix} y_1(t) \\ y_2(t) \end{bmatrix} \quad \alpha = \begin{bmatrix} \alpha_{10} \\ \alpha_{20} \end{bmatrix} \quad A = \begin{bmatrix} \alpha_{11} & \alpha_{12} \\ \alpha_{21} & \alpha_{22} \end{bmatrix} \quad \epsilon(t) = \begin{bmatrix} \epsilon_1(t) \\ \epsilon_2(t) \end{bmatrix}
  \]
Multivariate AR model

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

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

\[
\vdots
\]

\[
y_n(t) = \alpha_{n0} + \sum_{k=1}^{p} \alpha_{n1k} y_1(t-k) + \ldots + \sum_{k=1}^{p} \alpha_{nwk} y_n(t-k) + \varepsilon_n(t)
\]

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

\[
Y(t) = \alpha + \sum_{i=1}^{p} A_i Y(t-i) + \varepsilon(t)
\]

\[
\begin{bmatrix}
\alpha_{10} \\
\vdots \\
\alpha_{n0}
\end{bmatrix}
\begin{bmatrix}
y_1(t) \\
\vdots \\
y_n(t)
\end{bmatrix}
\begin{bmatrix}
\alpha_{11i} & \ldots & \alpha_{1ni} \\
\vdots & \ddots & \vdots \\
\alpha_{n1i} & \ldots & \alpha_{nni}
\end{bmatrix}
\begin{bmatrix}
\varepsilon_1(t) \\
\vdots \\
\varepsilon_n(t)
\end{bmatrix}
\]
VAR: convenient forms

Matrix form (hide ROIs) \( Y(t) = \alpha + A_1 Y(t-1) + \ldots + A_p Y(t-p) + \varepsilon(t) \)

Nice VAR(1) form (hide ROIs and lags): \( Z(t) = \nu + BZ(t-1) + u(t) \)

\[
Z(t) = \begin{bmatrix}
Y(t) \\
Y(t-1) \\
\vdots \\
Y(t-p+1)
\end{bmatrix} \\

\nu = \begin{bmatrix}
\alpha \\
0 \\
\vdots \\
0
\end{bmatrix} \\
B = \begin{bmatrix}
A_1 & \ldots & A_{p-1} & A_p \\
I_n & \ldots & 0 & 0 \\
\vdots & \ddots & \ddots & \vdots \\
0 & \ldots & I_n & 0
\end{bmatrix} \\
\varepsilon(t) = \begin{bmatrix}
\varepsilon(t) \\
0 \\
\vdots \\
0
\end{bmatrix}
\]

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

\[
Y = [Y(p+1), \ldots, Y(T)], \quad B = [\alpha, A_1, \ldots, A_p], \quad U = [\varepsilon(p+1), \ldots, \varepsilon(T)],
\]

\[
Z = \begin{bmatrix}
1 & 1 & \ldots & 1 \\
Y(p) & Y(p+1) & \ldots & Y(T-1) \\
\vdots & \vdots & \ddots & \vdots \\
Y(1) & Y(2) & \ldots & Y(T-p)
\end{bmatrix}
\]

Solve it with OLS:

\[
\hat{B} = YZ' = YZ'(ZZ')^{-1}
\]
VAR extended with covariates

- **Standard VAR($p$)** \[ Y(t) = \alpha + A_1 Y(t-1) + \ldots + A_p Y(t-p) + \varepsilon(t) \]
- **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($p$)**
  \[ Y(t) = \alpha + A_1 Y(t-1) + \ldots + A_p Y(t-p) + B Z_1(t) + \ldots + B_q Z_q(t) + \varepsilon(t), \]
  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 (1\textsuperscript{st} two tend to overestimate)
  - AIC: Akaike Information Criterion
  - FPE: Final Prediction Error
  - HQ: Hannan-Quinn
  - SC: Schwartz Criterion

- **Stationarity**: \( \text{VAR}(p) \ Y(t) = \alpha + A_1 Y(t-1) + \ldots + A_p Y(t-p) + \epsilon(t) \)
  - 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 $\geq$ 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
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![Image](http://xkcd.com/552/)
Other approaches

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