

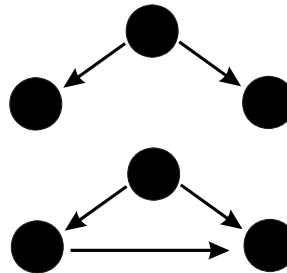
FUNCTIONAL VS. EFFECTIVE CONNECTIVITY

Functional connectivity:

- “temporal” correlation or covariance among measured neural elements
- requires no assumptions about mediation of influences

Effective connectivity:

- influence (effect) that one neural element has on another
- requires some assumptions about mediation of influences



Measures of functional connectivity cannot distinguish between the two networks above, effective connectivity can.

SOURCES OF VARIANCE

Across-task (within subject or averaged over subjects)

- Variance is introduced through the performance of several tasks. The simplest example would be the repetition of the same task several times. The variance would be due to the repetition of the task.
- In fMRI, this would be repetition of a type of event or block. You then compute subject-specific measures of functional or effective connectivity. The interpretation of the numbers indicates the degree to which areas within that subject are functionally linked, and may have a direct physiological translation.
- The difficulty with this source of variance is the generalizability of the subject's measures to the rest of the sample, or population.

Within-task, across subjects

- Variance is introduced by individual differences in how subject's brains respond to task demands.
- The interpretation of functional or effective connectivity in the case of across subjects patterns differs from above. Here we get an index of the reliability of functionally linkages across subjects and the sign of the linkage. The exact numerical value may not have a direct physiological interpretation.
- It needs to be emphasized that there need not be a decision made between the two sources of variance. Though they give different information, they give converging and complementary information.

STEP 1: REGION SELECTION

- You need to select a subset of maxima from contrasts for your path analysis.
- This can be based on both theoretical and data-driven constraints
- Note that as this is a modeling exercise, the anatomical model will not be a perfect representation of the way the brain really does the cognitive operation
- However, you need to make it very clear to the reader how regions were ultimately selected since that will help assess the utility of your model.
- **Number of regions, M, allowed in the model: $M = N - 1$**
- If more than one group, use the N of the smallest group
- If M is equal to or greater than N, the correlation matrix may be "rank-deficient" or "non-positive definite" (but this is not impossible to work around)

STEP 2: PREPARING ANATOMICAL MODEL

- **Connectivity:** You have already decided on the regions to include. Use neuroanatomical literature to determine which regions are connected and the direction(s) of this connectivity. Also, use theory to guide which connections you include in your model.

Anatomical references

- Note that while anatomical review papers are a good source for the connections, read them very carefully. Occasionally, a connection may be included in a system that is not well-documented or because the author of paper thinks it is there but no one else does. When in doubt, consult the original papers.
- Going from monkey anatomy to human can be tough, especially where Brodmann designation are not consistent between species. Petrides and Pandya (Petrides, M. & Pandya, D.N. in *Handbook of Neuropsychology* (eds. Boller, F. & Grafman, J.) 17-57 (Elsevier, Amsterdam, 1994) have redefined prefrontal cortex nomenclature (with mixed reviews) which helps to define homologous areas, and the mapping of posterior cortical visual areas seems to agree well (Sereni, M.I., *et al. Science* **268**, 889-893 (1995); DeYoe, E.A., *et al. Proc Natl Acad Sci USA* **93**, 2382-6 (1996); Felleman, D.J. & Van Essen, D.C. *Cereb. Cortex* **1**, 1-47 (1991).; Desimone, R. & Ungerleider, L.G. in *Handbook of Neuropsychology* (eds. Goodglass, H. & Damasio, A.R.) 267-300 (Elsevier, Amsterdam, 1989)).
- You can simplify the initial anatomical model to include only dominant connections and then add others based on the model fit (modification indices, see below) (e.g., McIntosh, A.R., *et al. J. Neurosci.* **14**, 655-666 (1994)). However, only add connections if they are anatomically feasible, otherwise they are hard to defend.

STEP 3: PREPARING FUNCTIONAL MODELS

- A functional model can either be in the form of a correlation or covariance matrix (Randy uses correlation matrices).
- You need one functional model for each condition and/or each group (e.g., one for specific AMs vs control tasks and one for general AMs vs fixation; one for LTLE AM retrieval and one for Control AM retrieval).

Extract the data:

- From what conditions or contrasts are you extracting data? (e.g., Specific AM vs. Control task and General AM vs. Control task?)
- Do you have different groups? (e.g., the same contrast -- AM vs. Control tasks -- for both the LTLE and control groups)
- Are you extracting data from an ROI (e.g., the signal averaged across a sphere using a program such as MarsBar) or the peak voxel in the region during some analysis? Does the voxel have to be the same in each condition as long as it is in the same proximity (i.e., anterior HC)?
- If data is being extracted from peak voxels, you can use the script: **SPM_msubj_mvox**

SPM_msubj_mvox (can be obtained from Donna Rose Addis, daddis@wjh.harvard.edu)

- Open SPM
- In matlab window, type **SPM_msubj_mvox**
- This will open SPM_get window
- Select the relevant **con00*.img** for each subject you wish to include.
- Press **Done**.
- Enter voxel cords (in mm): **Enter the MNI coordinates** for the voxels from which you wish to extract data. You may do this for as many voxels as required.
- Once finished, press **Enter** when prompted to enter another set of coordinates.
- Output: *msubj_mvox.txt* (rows = subjects; columns = voxels)
- NOTE: Rename the file with relevant info (e.g., condition and group), esp. if you are running this script again.

Create correlation matrix:

- Take your data from each subject (which should be one value for each region) and enter this into a data programme such as SPSS (with each region being a variable or column).
- Perform correlation analyses so that you end up with a correlation matrix of the correlations of data from each region with data from every other region (Note: the regions must be in the same order as your anatomical matrix).

STEP 4: DEFINING THE ANATOMICAL AND FUNCTIONAL MODELS

Defining the Anatomical model

- You need to prepare the anatomical model you are using in the form of a matrix so that you can enter **matrix locations** into the code to identify connected regions in your anatomical model
- e.g., your matrix should be set up so that “From” regions are columns and “To” regions are rows. If a connection exists from the region in Column 1 (e.g., LHC) and the region in Row 2 (e.g., RHC), enter a 1. If not, enter a 0. That way, when you come to write your anatomical model, it will consist of only those matrix locations which code for a connection (i.e., a “1”).

		FROM:			
		LHC	RHC	LPHG	LTPJ
TO:	LHC	-	1	1	0
	RHC	1	-	0	0
	LPHG	1	0	-	1
	LTPJ	0	0	1	-

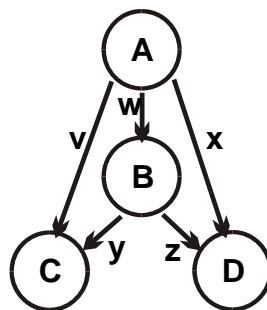
- Later, in the Lisrel code, when specifying a connection *to* RHC *from* LHC, you will refer to it as (2,1) as this connection is noted at row 2, column 1 of your matrix.
- Note:** The order in which your regions are listed in the anatomical must be the same as the order of regions in your functional models.

Defining the Functional model

- You simply use the correlation matrix created in step 3.

Structural equation modelling:

- You are basically treating the definition of a model in the same way as a typical regression analysis
- You set up a list of afferents for a given region, the afferents are “X” variables in a regression and the area is the “Y” variable
- Path analysis software differs in how these equations are specified. Although LISREL, AMOS etc. have a GUI that lets you draw the model, the GUI is cumbersome and it is worth using the scripting language for the program.
- Consider this simple network:



- The system can be expressed by a series of structural equations (regression equations) as follows:

$$A = \psi_A$$

$$B = wA + \psi_B$$

$$C = vA + yB + \psi_C$$

$$D = xA + zB + \psi_D$$

Where the symbol “ ψ ” denotes a residual influence unique to that variable (region).

- In path analysis programs, this is best expressed as a series of matrices:

$$\begin{matrix} \cdot & & \textcircled{R} & \cdot & & | \\ \begin{pmatrix} A \\ B \\ C \\ D \end{pmatrix} & = & \begin{pmatrix} 0 & 0 & 0 & 0 \\ w & 0 & 0 & 0 \\ v & y & 0 & 0 \\ x & z & 0 & 0 \end{pmatrix} & * & \begin{pmatrix} A \\ B \\ C \\ D \end{pmatrix} & + & \begin{pmatrix} \psi_A & 0 & 0 & 0 \\ 0 & \psi_B & 0 & 0 \\ 0 & 0 & \psi_C & 0 \\ 0 & 0 & 0 & \psi_D \end{pmatrix} \end{matrix}$$

Where the symbols above are the matrix names in LISREL terminology.

- All programs attempt to estimate the values for the path coefficients and residuals to reproduce the original covariances among the regions.
- Let’s make the covariance matrix among regions A, B, C, & D = \textcircled{R} . Then in LISREL, the covariance matrix is fit through a series of iterations based on the following formula:

$$\sigma = \text{inv}(I - \beta)^T * (\psi^T * \psi) * \text{inv}(I - \beta)$$

where *inv* denotes matrix inversion, *I* in an identity matrix, and *T* denotes a matrix transpose.

- There are several iterative methods that have been used. No matter which one is used, if it is an iterative method there is no guarantee that the solution reached is unique, no matter what anyone tells you. You can ensure that the fit is close to optimal, but that’s about it.

STEP 5: STRUCTURAL EQUATION MODELLING

Omnibus test: Null vs. Alternative

- In most models the first step is to determine if there are any differences in path coefficients between tasks/groups. This is like an ANOVA where you have more than two levels of the independent variable – you first run the ANOVA to see if there are differences then do some sort of follow-up tests to tease the differences apart.
- The omnibus test involves two models.
 - The null model states that there are no differences in path coefficients. All coefficients are constrained to have equal estimates. This is usual a sort of "average" path coefficient.
 - In the alternative model, the constraints are removed.

- To compare the two models you take the chi-squared goodness-of-fit index for the null model and subtract the chi-squared value for the alternative model. You do the same for the degrees of freedom for the two models. The difference in chi-squared is evaluated in a chi-squared table with the degrees of freedom equal to the difference in the degrees of freedom between null and alternative models.

Testing Individual paths

- To determine which of the path coefficients are most different, you test each individual path, in the same way that you compare null and alternative models you can compare individual coefficients.
- Start with the path of interest constrained, obtain the chi-squared, then run a model with the path unconstrained and compare the change in chi-squared. If the change is significant, leave the path unconstrained and continue testing.
- As with the hierarchical models, in some cases the order you test may give you somewhat different outcomes, especially in models with a lot of reciprocal connections. You would need to re-run the tests in a different order to ensure stability of the results.
- One obvious problem with this is the potentially large number of coefficients to test and potential for Type I error. To minimize this, you can test coefficients in blocks like efferents/afferents for an area and correct the alpha for the number of areas rather than the number of path coefficients.

Single group multiple tasks

- The process for this is rather straightforward. You step through the null and alternative models and then assess individual paths if needed. Certain tasks can be constrained to equal one another if there is good reason to expect such equalities.

Multiple group multiple tasks

- First off, you MUST have the same anatomical model between groups. It is very difficult to defend a group difference when the anatomical models are different to begin with. One option, if there is ambiguity in the anatomical model, is to combine the model between groups to ensure that the same network is being tested.
- The process of testing gets a little more complicated. Once the omnibus is done and if you want to test specific tasks/paths you may either proceed by pairwise group comparison for each task or test for task differences within group. The process will depend on the particular data set in many cases. You can step through the model by first testing main effects (group, task) then the interactions (group by task).

Assessment of the model

- Once you have a reasonable model, there may be a need to "clean it up" if there are excessively large path coefficients ($\ll 1$) or instabilities. Path coefficients that are greater than one cannot, in theory, exist (this would mean there is more variance to account for than a region has), so you need to impose a constraint by fixing the coefficient to some value under one. These high estimates arise when there are several larger correlations and/or reciprocal connections.

Stability index

- This number is derived from the total effects computation.
- A stability index much greater than one means the model is *not stable* because of high indirect effects. These usually come from either paths greater than one and many reciprocal connections.
- Fixing coefficients or imposing equality constraints within loops will help bring the index down

NB: If you make a drastic change to the model, you will have to go back and reassess the statistical significance.

Modification index

- This is computed for all fixed and constrained coefficients in the model (paths and residuals).
- They represent the expected improvement in fit if the parameter is free and can be interpreted as a chi-square with one degree of freedom.
- If there is a high index for an anatomically feasible path it may be included in the model, but if this is done after statistical testing, you will need to go back and do it again.

Interpretation

- Obtaining the total effects in the final output will help in the ultimate interpretation and graphing the models is definitely useful to see what's going on.
- When looking through these models, pay special attention to the overall pattern rather than a particular path. Trying starting at one node then move around the model as if you were following information flow.

PATH ANALYSIS – CODE FOR LISRELEXAMPLE OF A NULL MODEL (ANNOTATED)

** Note this is made-up dataset**

Control_Subjects [title line]

da [datacard] no=12 [# observations/subjs] ni=4 [# input variables/regions] ma=km
[matrix=correlation] ng=2 [# groups/conditions]

km [correlation matrix – this is your functional model; an also be covariance matrix or “cm”]

* [free format]

1

0.493 1

0.187 0.151 1

0.162 0.139 0.073 1

la [labels for regions ****must be in the order of appearance in correlation matrix**]

lmpfc rmpfc lhc rhc [labels]

mo [model line] ne=4 [# variables] be=fi,fu [beta matrix is a fixed, full matrix] ps=fi,di [psi is a fixed, diagonal matrix]

[specification of anat connections (anat model): to row, from column]

fr [free, i.e., not zero] be(1,2) [to region 1 from 2]

fr be(2,1) [to region 2 from region 1]

fr be(1,3)

fr be(3,1)

fr be(1,4)

va 0.4 [reisdual value set at 0.4 is rule of thumb] ps(1) -ps(4) [residual regions 1-4]

ou [output]

LTLE_Subjects

da no=12 ni=4 ma=km

km

*

1

0.442 1

0.014 0.576 1

0.257 0.086 0.028 1

la

lmpfc rmpfc lhc rhc

mo [model line] be=ps [beta=psi] ps=in [psi=invariant, i.e, beta has same pattern and starting values as model 1]

eq be(1,1,2) be(1,2) [i.e., set be(1,2) to be the same as be(1,2) from condition 1, ie, be(1,1,2)]

eq be(1,2,1) be(2,1)

eq be(1,1,3) be(1,3)

eq be(1,3,1) be(3,1)

eq be(1,1,4) be(1,4)
 ou [output] mi [modification indices] ss [standardised solution] ef [effects deconvolution]

PATH ANALYSIS – CODE FOR LISREL

NULL MODEL

Control_Subjects

da no=12 ni=4 ma=km ng=2

km

*

1

0.493 1

0.187 0.151 1

0.162 0.139 0.073 1

la

lmpfc rmpfc lhc rhc

mo ne=4 be=fi,fu ps=fi,di

fr be(1,2)

fr be(2,1)

fr be(1,3)

fr be(3,1)

fr be(1,4)

va 0.4 ps(1) -ps(4)

ou

LTLE_Subjects

da no=12 ni=4 ma=km

km

*

1

0.442 1

0.014 0.576 1

0.257 0.086 0.028 1

la

lmpfc rmpfc lhc rhc

mo be=ps ps=in

eq be(1,1,2) be(1,2)

eq be(1,2,1) be(2,1)

eq be(1,1,3) be(1,3)

eq be(1,3,1) be(3,1)

eq be(1,1,4) be(1,4)

ou mi ss ef

PATH ANALYSIS – CODE FOR LISREL**ALTERNATE MODEL (ANNOTATED)**

[First functional model entered exactly as in code for Null Model]

```
Control_Subjects
da no=12 ni=4 ma=km ng=2
km
*
1
0.493 1
0.187 0.151 1
0.162 0.139 0.073 1
la
lmpfc rmpfc lhc rhc
mo ne=4 be=fi,fu ps=fi,di
fr be(1,2)
fr be(2,1)
fr be(1,3)
fr be(3,1)
fr be(1,4)
va 0.4 ps(1) -ps(4)
ou
```

[Remainder of functional models differ – see notes below]

```
LTLE_Subjects
da no=12 ni=4 ma=km
km
*
1
0.442 1
0.014 0.576 1
0.257 0.086 0.028 1
la
lmpfc rmpfc lhc rhc
mo be=ps ps=in
```

[Note: “eq” lines have been commented out, thus, all connections are free to vary in both condition]

```
!eq be(1,1,2) be(1,2)
!eq be(1,2,1) be(2,1)
!eq be(1,1,3) be(1,3)
```

!eq be(1,3,1) be(3,1)
!eq be(1,1,4) be(1,4)
ou mi ss ef

(8) PATH ANALYSIS – OUTPUT FROM LISREL

- Firstly, check the **overall significance** of models
 - Scroll through output of the Null and Alt models until you find the “Goodness of Fit Statistics”

```

Goodness of Fit Statistics
Degrees of Freedom = 185
Minimum Fit Function Chi-Square = 692.64 (P = 0.0)
Normal Theory Weighted Least Squares Chi-Square = 1796.03 (P = 0.0)
Contribution to Chi-Square = 210.32
Percentage Contribution to Chi-Square = 11.71
Estimated Non-centrality Parameter (NCP) = 1611.03
90 Percent Confidence Interval for NCP = (1478.92 ; 1750.56)

```

- For each, note down the values of the first two lines: df and the “minimum fit function Chi Square”
- Using excel, determine the difference between the chi values and the df. Use the CHIDIST function in Excel to determine the significance (p value) of your Difference Chi Square
- e.g., if your Chi diff value is in cell A1 and Chi diff df in cell B1, in cell C1, enter formula: =CHIDIST(A1,B1) and it will return the p value

	A	B	C	D	E	F	G	H
1	FILE	CONNECTION	FREE TO VARY	MODEL	CHI	df	pvalue	
2	1	All - omnibus			null	397.57	107	
3					alt	309.26	58	
4						88.31	49	0.00049037

- Check the **stability** of the models
 - Scroll through output until you find:

```

Largest Eigenvalue of B*B' (Stability Index) is 0.373

```

- There will be one of these lines for each functional model (its given between the Total effects and Indirect effects)
- This value indicates the stability of each functional model. If the value is under 1, the model is stable. If it is over 1, the model is not stable and (I think) the next step is to look over the modification indices to see how things would change when certain connections are removed.

PATH ANALYSIS – WHICH CONNECTIONS ARE SIGNIFICANTLY DIFFERENT?

- If there is an overall significant difference between the Null and Alt models, you then have to determine where the differences lie. In other words, which connections in your model are contributing to this overall difference.
- This is done by repeating the test of the Alt model repeatedly, where each time you let a different connection vary
- Use Alt Model Code from before, but this time, comment out only one connection.

e.g., In any models after the first functional model, enter your connections with ! for the connection to set as varying:

```
!eq be(1,1,2) be(1,2)
eq be(1,2,1) be(2,1)
eq be(1,1,3) be(1,3)
eq be(1,3,1) be(3,1)
eq be(1,1,4) be(1,4)
eq be(1,4,1) be(4,1)
eq be(1,1,6) be(1,6)
eq be(1,6,1) be(6,1)
```

- Run the test, enter Chi square & df into Excel and see whether it contributes to significance of the difference between Null and Alt (see example on next page)
- If it does, leave that connection commented out, and comment out the next.
- If it doesn't, remove the ! from that connection (which means it will no longer be free to vary), and comment out the next connection, and re-run.

	A	B	C	D	E	F	G	H	I	J
1	FILE	CONNECTION FREE TO VARY			MODEL	CHI	df	pvalue	diff in p	SIG?
2	1	All - omnibus			null	397.57	107			
3					alt	309.26	58			
4						88.31	49	0.00049037		
5	Pairwise tests: Emt-Sig									
6	2	To RMPFC from RTpole: be(2,5)								
7					null	397.57	107			
8					alt	387.04	106			
9						10.53	1	0.00117452		SIG
10	3	To LMPFC from REmtACC: be(1,3)								
11					null	397.57	107			
12					alt	386.46	105			
13						11.11	2	0.00386807	-0.0026935	NS
14	4	To REmtACC from RMPFC : be(3,2)								
15					null	397.57	107			
16					alt	384.57	105			
17						13	2	0.00150344	-0.0003289	NS
18	5	To RMPFC from REmtACC: be(2,3)								
19					null	397.57	107			
20					alt	385.36	105			
21						12.21	2	0.00223168	-0.0010572	NS
22	6	To RdmTHAL from LMPFC: be(4,1)								
23					null	397.57	107			
24					alt	381.29	105			
25						16.28	2	0.00029164	0.00088288	SIG

← Significant connection (Column H)

← Insignificant connection as doesn't contribute to p-value as indicated by negative value in Column I

← Significant connection as this increase the significance in the difference between Null & Alternate models (Column I)