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# Multivariate Statistical Analysis in fMRI

*A Survey of the Basic Theory*

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In functional magnetic resonance imaging (fMRI), the question of whether a region of the brain is being activated with a stimulus is important. Typically, it is determined whether individual voxels are active through a multiple regression and thresholding [1]–[3] with statements made about clusters of voxels. Standard individual voxel activation statistics are computed via multiple regression. The generally computed activation  $t$ -statistic maps are thresholded objectively with methods that account for the multiple comparisons problem such as Bonferroni adjustment for controlling the familywise error rate. Regions of interest (ROIs) are formed from the thresholded maps by using the AND in addition to the OR operation [4]. The voxels in the ROIs can be analyzed for joint activation. It is extremely important to determine appropriate thresholds to determine clusters of voxels to comprise ROIs [5].

## Regression Analysis

The multivariate fMRI regression model [4], [5] is a generalization of the univariate regression model from a single voxel to  $p$  voxels. The model is  $Y = XB + E$ , where  $Y$  is an  $n \times p$  matrix of observed voxel time courses with the  $j$ th column  $Y_j$  being the observed time series in the  $j$ th voxel;  $X$  is an  $n \times (q + 1)$  design matrix that contains any regressors such as an intercept, a time trend, and a reference function;  $\beta$  is a matrix of regression coefficients with the  $j$ th column  $\beta_j$  being for the  $j$ th voxel; and  $E$  is a matrix of error terms with the  $j$ th column  $E_j$  the errors for a given voxel time course or, alternatively, the rows of  $E$ ,  $\varepsilon_j \sim N(0, \Sigma)$ , where  $\Sigma$  is the spatial covariance matrix between voxels. Given this model and a specification that the errors are normally distributed and temporally independent, the maximum likelihood estimator (MLE) of  $\beta$  is  $B = (X'X)^{-1}X'Y$ . Note that this estimator does not depend on the spatial covariance or correlation between the voxels. Within this framework, the estimated regression coefficients for the  $j$ th voxel is the  $j$ th column of  $B$ . Furthermore, the MLE of  $\Sigma$  is  $S = (Y - XB)'(Y - XB)/n$ . The estimated covariance matrix  $S$  is not of full rank  $p$  but is of rank  $n - q - 1$ . Bayesian methods can be implemented in which the estimator of the covariance matrix is positive definite [3].

The usual method to determine brain activation is to consider each voxel independently of the others. As previously men-

tioned, the design matrix contains a reference function that is a representation of the response we seek. A measure of association between this reference function and the time course in each voxel can be found by looking at the significance level of the coefficient associated with the reference function. In voxel  $j$ , the hypothesis that a coefficient (for example, the last element of  $\beta_j$ )  $\beta_{qj} = 0$  can be tested with

$$t_j = b_{qj} / [nW_{qq}S_{jj} / (n - q - 1)]^{1/2},$$

where  $W = (X'X)^{-1}$ ,  $W_{qq}$  is its last diagonal element, and  $S_{jj}$  is the  $j$ th diagonal element of the  $S$  voxel. By a priori specifying a level of significance of  $t_j$ , which has a student  $t$  distribution with  $n - q - 1$  degrees of freedom such as  $\alpha = .05$ , critical cutoff values  $\pm t_{n-q-1, \alpha/2}$  can be determined. If the voxel  $t$ -statistic falls within  $\pm t_{n-q-1, \alpha/2}$ , then the voxel is deemed not active and assigned the anatomical grayscale value, while if it is deemed active, it is colored accordingly. However, with the number of voxels, this threshold will result in a large number of false positives ( $\alpha p$  on the average) strictly by chance. Generally, this threshold is adjusted for the number of voxels with a method such as the Bonferroni correction, where the voxelwise significance is changed from  $\alpha$  to  $\alpha^* = \alpha/p$  as discussed in [2].

## Principal Component Analysis

Principal component analysis (PCA) is a statistical method to determine components that are linear combinations of the original variables that sequentially maximize the variance of the weighted sum [7]. In PCA, the first principal component is the linear combination of the variables with maximal variance that is the direction within the original data with the largest variability. The second component is the linear combination with the next largest variability that is orthogonal to the first. Further components are the linear combinations that maximize the variability and are orthogonal to all previous ones. The number of components is equal to the number of original variables  $p$ . In PCA, the population covariance matrix  $\Sigma$  is estimated by its sample value  $S = (Y - XB)'(Y - XB)/n$  and the orthogonal components computed from  $S$ .

The output of a PCA is a square matrix  $W$  of dimension  $p$  such that  $D_\lambda = WSW'$ , where the rows of  $W$  are orthonormal

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vectors (eigenvectors), each sequentially maximizing the percent of variation, and  $D_\lambda$  is a diagonal matrix of component variances (eigenvalues), each denoted with a subscripted  $\lambda$ . Algebraically speaking, the matrix  $W = (w_1, \dots, w_p)'$  is determined so that the components or linear combinations  $Wx_j$ ,  $j = 1, \dots, p$  with variance given by  $\text{var}(Wx_j) = w_1' \text{var}(x_j) w_1 = w_1' S w_1$  of the original variables determined.

The vector  $w_1$  is now determined to be that value that maximizes the variance subject to  $w_1' w_1 = 1$ . The method of Lagrange multipliers is applied

$$\partial/\partial w_1 [w_1' S w_1 - \lambda_1 (w_1' w_1 - 1)] = 2 S w_1 - 2 \lambda_1 w_1 = 0,$$

which is reexpressed as

$$(S - \lambda_1 I_p) w_1 = 0,$$

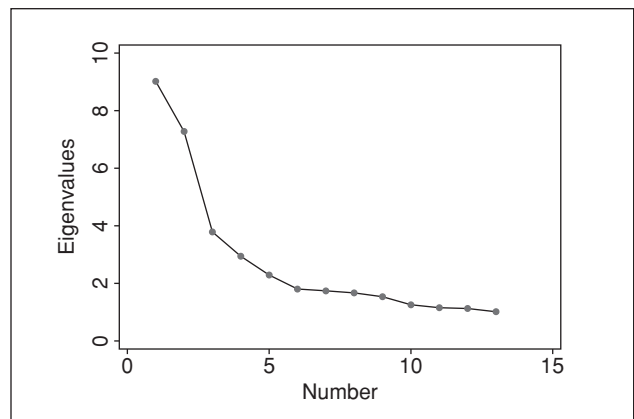
and since  $w_1 \neq 0$ , there can only be a solution if

$$|S - \lambda_1 I_p| = 0.$$

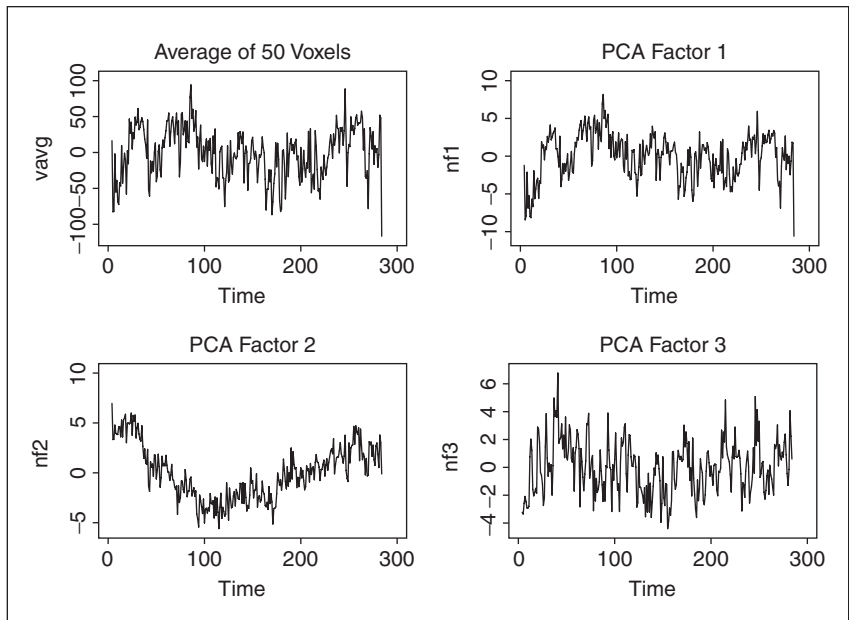
It is apparent that  $\lambda_1$  must be a latent root of  $S$ , and  $w_1$  is a normalized latent vector of  $S$ . There are  $p$  such latent roots that satisfy the equation. The largest is selected. The other rows of  $W$  are found in a similar fashion. In practice, PCA is used as a dimension reduction method. Since a small number of components account for the majority of the variability in the data, they are retained, and the others discarded.

PCA can be applied to a set of voxels to create an ROI and extract common information. For example, a rapid visual information processing (RVIP) task [8], [9] was used to examine the effects of nicotine (patch and placebo patch) on sustained attention. The RVIP task was alternated with a control task and a short rest period. Applying PCA to 50 neighboring voxels in this ROI produces 14 components with an eigenvalue greater than one. Their components account for 75% of the variation in the data. The graph in Figure 1, the scree plot, displays the eigenvalues. The majority of the explained variance is in the first three components (40%). Figure 2 displays the first, second, and third components of the PCA analysis, as well as the average of the 50 voxels. As is often the

case, the first principal component is the largest source of variation in the data and is nearly the same as the temporal mean. The first principal component within an activated region is very similar across subjects; the other components vary from subject to subject (data not shown). The second component appears to be related to the trend (or drift) in the ROI. The temporal drift is often the second-largest source of



**Fig. 1.** Graph of the magnitude of the eigenvalues. The largest are the first two; the corresponding components account for 32% of the variability.



**Fig. 2.** Graph of the average of 50 voxels in an ROI together with the first three eigenvectors of the PCA of the same 50 voxels.

variation. The third component contains some of the high-frequency noise that is still present in the averaged response but not in the first principal component. The higher-order components are difficult to interpret in most fMRI studies (Figure 3), although they can be used to differentiate subgroups of subjects. In Figure 4, the two nicotine usage subgroups have very similar first principal components but quite different second principal components. In addition,

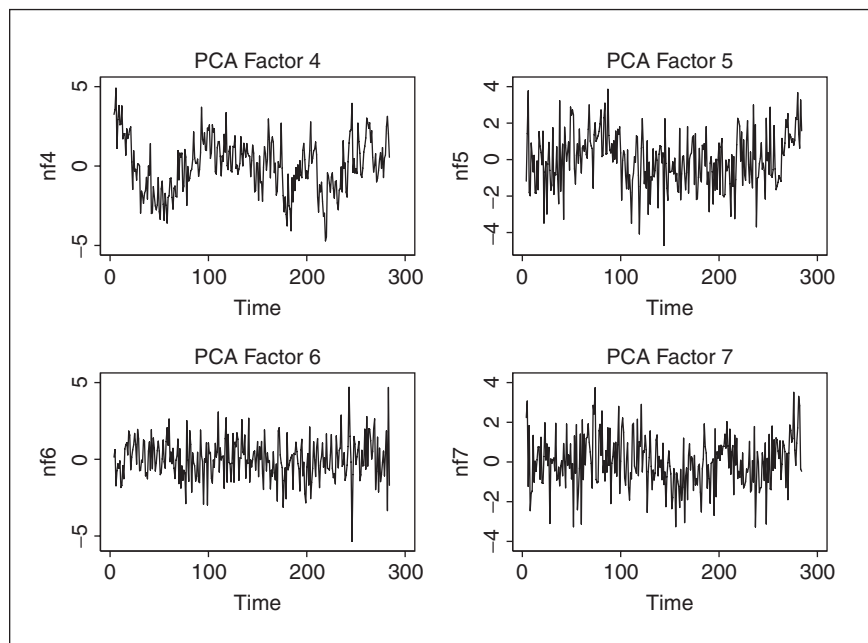
since the ROIs often have different nonlinear temporal trends (usually curvilinear polynomials of order 2 and higher), PCA applied to the set of ROIs will often group them into components that have the same relationship to task but different temporal trends (Figure 5). Software for PCA is contained in nearly all data analysis packages, e.g., SAS, Stata, SPSS, R, MATLAB, Statistica.

As stated, PCA determines orthogonal components or sources of variation. Other methods are in use to extract sources such as factor analysis, independent component analysis, and Bayesian source separation [10].

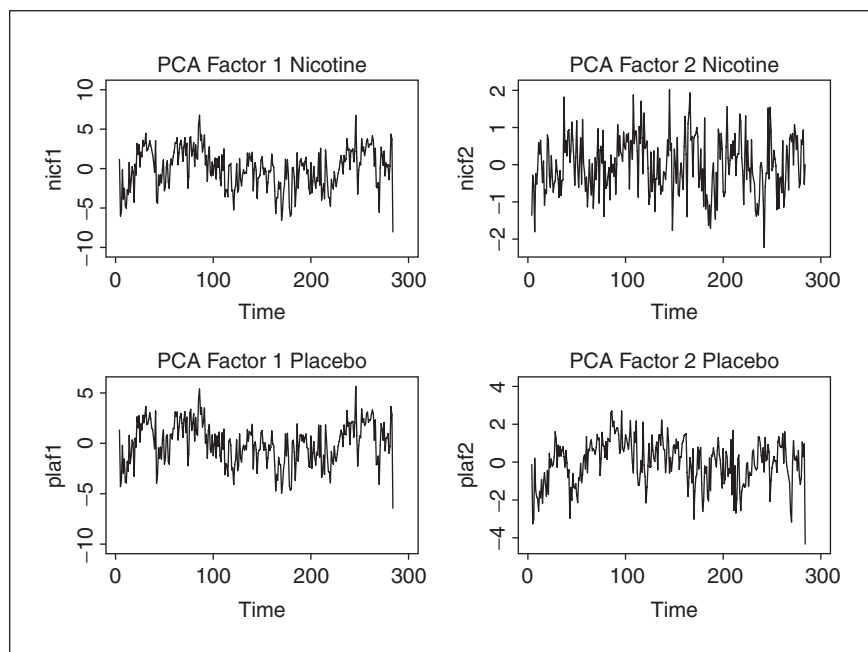
### Connectivity Analysis

In fMRI, the question of whether two or more regions of the brain are associated with each other is important.

Functional connectivity is the determination of the activation pattern of the blood-oxygen-level-dependent (BOLD) response to a task. Effective connectivity is the determination of the strength of the interconnections between functionally active regions of the brain. Effective connectivity analysis allows testing of whether a neural network changes in its connections between sites. Changes in the connection strength between regions in the brain can occur independently of changes in response magnitude and extent in the region. Consequently, changes in performance can be ascribed to changes in the region or changes in how the regions inter-communicate. A region's connectivity changes may precede the activity changes, follow the activity changes, or not change at all. Any of these conclusions help to explain the process of changes in the network. Structural equation modeling (SEM) is the technique used to estimate the strength of the paths between different brain regions. McIntosh and Gonzales-Lima [11], [12] first used SEM to study the connections in the rat visual cortex. In later work, Honey [13] used SEM to examine the effects of verbal working memory load on intercortical connectivity. Buchel and Friston [14] extended the SEM method to examine modulation of the connectivity between the visual cortex and the posterior parietal by the level of activity of the prefrontal cortex. They used interactions and nonlinear terms in the SEM model. Couill and Buchel [15] extended this idea to the modulation of connectivity by external factors, namely, the usage of different doses of clonidine, a D2 agonist.



**Fig. 3.** Graph of the fourth through seventh eigenvectors of the PCA of the 50 voxels. As the eigenvalues decrease in magnitude and the components account for less of the variance, the components appear to be accounting for high-frequency noise.

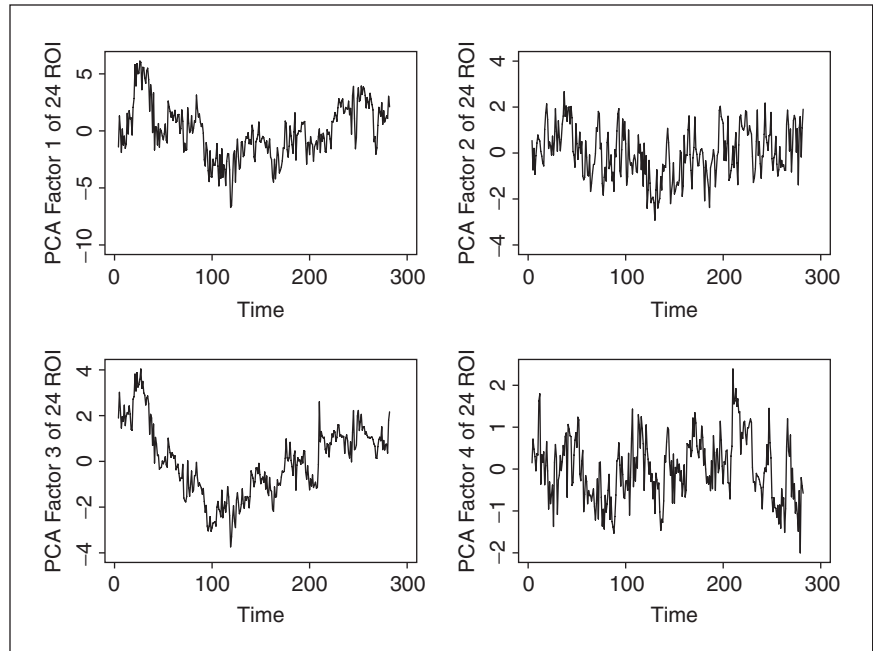


**Fig. 4.** A graph of the two major eigenvectors of two separate PCA analyses on the same 50 activated voxels in subjects on a nicotine patch 2 h after their last cigarette and the same subjects on a placebo patch 2 h after their last cigarette.

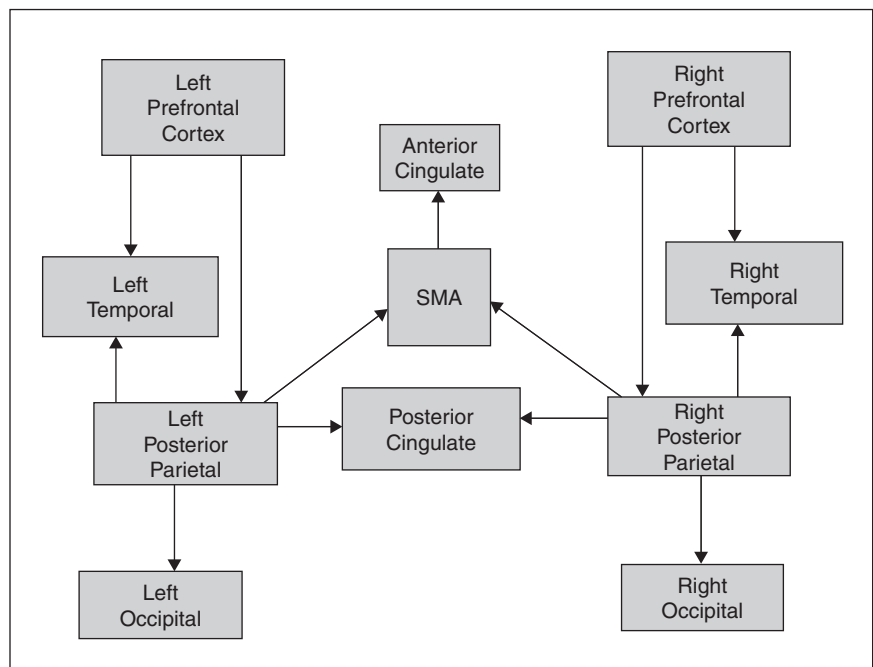
SEM modeling starts with an a priori model of the regions involved in the neural network and the connections between them based on known anatomical connections between ROIs and their direction (bidirectional connections are allowed). Each ROI defines a regression equation relating its pattern of response to the responses in the ROI connected to it. The simultaneous system of equations is solved via least squares or maximum likelihood for the strengths of the connections (the path coefficients) joining the regions. The standardized path coefficients can be interpreted as partial correlation coefficients or as partial regression coefficients. Changes in the estimated connection strengths can be tested with Wald tests, while overall goodness of fit is assessed with chi-squared comparisons of the covariance matrix from the a priori theoretical model and the correlation matrix of the observed data [16]. Split-half and, more recently, jackknife methods are used to validate the results across participants [17]. The data for an SEM analysis is derived from the fMRI time series after motion correction and detrending. Whether the fMRI study is a block design or an event-related design, the model for the neurophysiological connections is based on animal and lesion studies.

For example, we can determine effective connectivity changes in regions activated by the RVIP task [8] with real and placebo nicotine patches 2 h after application. The first step is to identify the regions that should be involved in the sustained attention/working memory RVIP task. The model for the expected connections is based on Kolb and Whishaw's [18] discussion of regions involved in sustained attention. The anatomic connections expected are displayed in Figure 6. Data was processed with AFNI 2.2 after performing motion correction and edge detection. Functional ROIs were defined in standard stereotaxic space by regions differentially activated in the non-smokers [9]. The path coefficients were estimated separately for each condition, as a system of regression equations using SAS 8.2 proc callis. Table 1 displays the results for some of the regions [19]. Statistical comparisons of the individual connections between conditions were made using Wald tests, *t*-tests using the difference in the connections divided by the standard error of the difference. Most

notable are 1) the strengthening of the path coefficients between the supplemental motor area (SMA) and the anterior cingulate, suggesting possible increases in attention to emotions and external processes when on the placebo, and 2) the weakening of the path coefficients between the prefrontal cortex and the posterior parietal region, suggesting possible difficulties with goal directed tasks and attention.



**Fig. 5.** Graph of the four main eigenvectors of a PCA analysis of the mean responses of 24 active ROI. The basic task pattern is similar in all the eigenvectors, but the nonlinear trend differs among the eigenvectors, corresponding to different nonlinear trends in some of the ROI.



**Fig. 6.** The expected connection pattern of the regions activated by the RVIP sustained attention and working memory task. The diagram is based on animal studies and human lesion studies.

**Table 1. Path coefficients for selected connections in the RVIP task.**

Regions that Decrease Connectivity	Nicotine	Placebo
Left posterior parietal to hippocampus	0.15	0.06*
Prefrontal to left posterior parietal	0.36	0.24**
Prefrontal to right posterior parietal	0.36	0.23**
Regions that Increase Connectivity		
Left posterior parietal to temporal	0.16	0.29**
SMA to anterior cingulate	0.12	0.25**
Right Posterior parietal to SMA	0.13	0.22*
Right posterior parietal to visual	0.15	0.22*

\* $P < 0.05$ , \*\* $P < 0.01$

The path coefficients were estimated separately for the nicotine patch and placebo patch conditions. Comparisons between conditions used a paired *t*-test.

Some general-purpose software packages can be used for SEM, namely SAS, Statistica, and R. There are also special-purpose packages for estimating the path coefficients from SEM; the best known is the package LISREL, which allows the problem to be specified either as a set of simultaneous regression equations or as a set of correlation matrices.

### Conclusions

Multivariate regression analysis with multiple comparisons corrections allows the determination of activated voxels that can then be grouped into ROIs. PCA is useful in extracting common temporal response features of an ROI as well as differentiating the temporal response of groups of commonly responding ROI. It can also be used to examine differences in the temporal response of subgroups of subjects in the study. SEM is a technique that requires a priori knowledge of the connections and their direction between ROIs. It is particularly useful in identifying changes in connectivity that result from different interventions or different classes of patients.

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