

Models and Applications of Multivariate Statistical Analysis in fMRI

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1. Introduction

In functional magnetic resonance imaging (fMRI), the question of whether a region of the brain is being “activated” or statistically significantly associated with a pre defined reference wave form characterizing the presentation of an experimental “stimulus” condition is important. Typically, it is determined whether individual voxels are active through a multiple regression and thresholding ([1], [2], [3]) with statements made about clusters of voxels. Standard individual voxel activation statistics are computed via multiple regression. Activation “t-statistic maps” which are a map generated from a t-statistic derived from a hypothesis test performed in each voxel are computed. A “thresholded” or map-wise critical value is objectively determined with methods that account for the multiple comparisons problem such as Bonferroni adjustment for controlling the family-wise error rate or the Benjamini-Hochberg adjustment for controlling the false-discovery error rate [3]. Regions of interest (ROIs) are often formed from the thresholded maps of a group of subjects by using the “and” in addition to the “or” operation on a voxel-wise basis after coregistration. The voxels in the ROIs can be analyzed for joint activation [4, 5]. It is extremely important to determine appropriate thresholds to determine clusters of voxels to comprise ROIs [6].

2. Regression Analysis

The multivariate fMRI regression model ([7], [8]) is a generalization of the univariate regression model from a single voxel to p voxels. The model is $Y = X\beta + E$, 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, β is a $(q+1) \times p$ matrix of regression coefficients with the j^{th} column β_j being the those for the j^{th} voxel, E is a $n \times p$ 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 Σ is the spatial covariance matrix between voxels. Given this model and a specification that the errors are normally distributed and temporally independent or suitably prewhitened [9], the maximum likelihood estimator (MLE) of β is $B = (X'X)^{-1}X'Y$. Note that this estimator with the same design matrix for all voxels 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 . Further, the MLE of Σ is $S = (Y - X B)'(Y - X B)/n$. 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 [10].

The usual method to determine brain activation is to consider each voxel independently of the others. As previously mentioned, 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 observed 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 β_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 S voxel. By *a priori* specifying a level of significance of t_j such as $\alpha=.05$ which has a standard student t distribution with $n-q-1$ degrees of freedom or $n-\text{rank}(X)$, under the null hypothesis, 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 grey scale value, while if it above the critical cutoff then it is deemed active and is colored accordingly. However, with the number of voxels, this “threshold” will result in a large number of false positives strictly by chance (αp on the average under the null hypothesis). Generally this threshold is adjusted for the number of voxels with a method such as the Bonferroni correction where the voxel-wise significance is changed from α to $\alpha^* = \alpha/p$ as discussed in [3]. If the number of voxels were *a priori* reduced to a small group say p_{ROI} , then overall ROI activation can be determined with a contrast, say $C=(0, \dots, 1)$ and

$$F_q = \{(n-q-p_{\text{ROI}})B_q [(Y-X B)'(Y-X B)]^{-1} B_q'\} / \{p_{\text{ROI}} W_{qq}\}$$

where B_q is the $(q+1)$ st row of B . The statistic F_q has an F distribution with p_{ROI} numerator and $n-q-p_{\text{ROI}}$ denominator degrees of freedom. This method determines if the voxels in the ROI are active together versus not active together. If this statistic is significant, then it should be followed up with individual voxel-wise tests as above similar to individual tests for differences in means after an ANOVA. It may be the case, for example with two voxels that this F statistic is significant, which when followed up by individual voxel-wise tests reveal that this is from one voxel being very significant while the second is not or from two voxels that are slightly below individual significance [4].

3. 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 linear combination ([8],[11]). In PCA, the first principal component is the linear combination of the variables with maximal variance which 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 Σ is estimated by its sample value $S=(Y-X B)'(Y-X B)/n$ and the orthogonal components computed from S . If the design matrix contains the reference function, then the residual will contain little information about the signal of interest. The PCA is generally performed on an S matrix where there is no reference function contained in X .

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 vectors (eigenvectors) each sequentially maximizing the percent of variation and D_λ is a diagonal matrix of component variances (eigenvalues), λ 's. Algebraically speaking, the matrix $W=(w_1,\dots,w_p)'$ is determined so that the components or linear combinations Wx_j , $j=1,\dots,p$ have variance given by $\text{var}(Wx_j) = w_j' \text{var}(x_j) w_j = w_j' S w_j$. The number of meaningful components extracted with positive eigenvalues is less than p and equal to the rank of S .

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 re-expressed 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 λ_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 from an ROI and extract common information. For example, a rapid visual information processing (RVIP) task ([12],[13]) 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 one of the ROIs produces 14 components with an eigenvalue greater than one. These 14 components account for 75% of the variation in the data.

The graph in Figure 1, a scree plot, displays the sequentially decreasing normalized eigenvalues. The majority of the explained variance is in the first three components (40% of the 75%). Figure 2 displays the average time course of the 50 voxels and the first three components or PCA factors. The first principal component is the largest source of variation in the data and as is often the

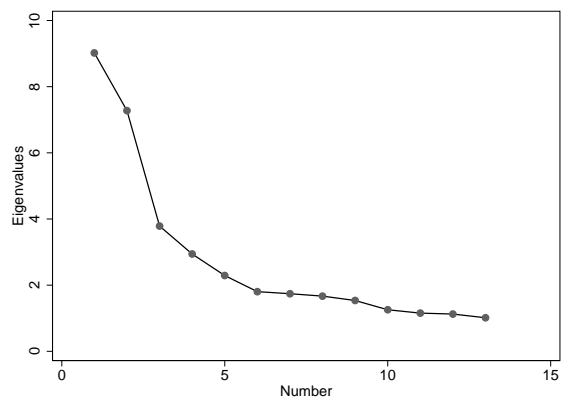


Figure 1. Graph of the magnitude of the eigenvalues. The largest are the first two; the corresponding components account for 32% of the variability.

case captures much of the temporal structure and looks similar to the temporal mean.

In a block design it is often dominated by a periodic trend that corresponds to the alternation of the tasks [26]. 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. Often the temporal trends are non-linear (usually curvilinear polynomials of order 2 and higher).

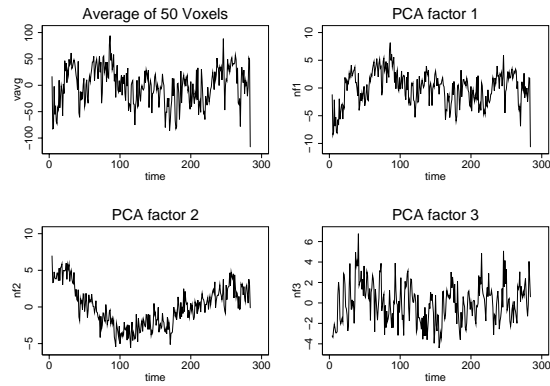


Figure 2. Graph of the average of 50 voxels in an ROI together with the first three eigenvectors of the PCA of the same 50 voxels.

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. By using them in a subsequent regression analysis they may serve to identify the differences among the group response to the task.

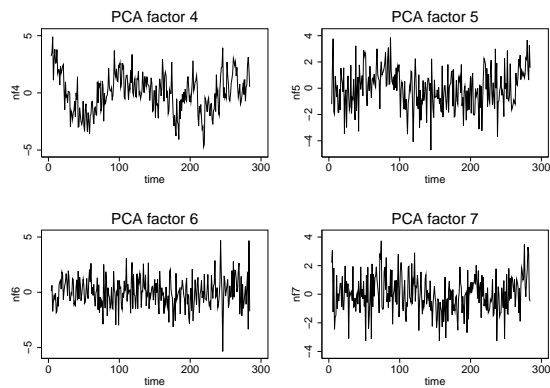


Figure 3. Graph of the fourth through seventh eigenvectors of the PCA of the 50 voxels. As the eigenvalues decrease in magnitude and the components account for less of the variance; the components appear to be accounting for high frequency noise.

For example, in Figure 4 the two nicotine usage subgroups have very similar first principal components, but quite different second principal components. Each ROI often has its own temporal trend which sometimes becomes the second principal component. Visually, the rest of the components for each of the ROIs will often have a similar relationship to the underlying boxcar task (Figure 5).

Software for PCA is contained in nearly all data analysis packages: SAS, Stata, SPSS, R, Matlab, Statistica, etc. 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 ([11], [12], [14]).

Historically, PCA was first used in PET [15] to identify connections between different regions of the brain. With PET, unlike fMRI, the time series were very short; however, the PCA was still applied to all the voxels ($10^4 - 10^5$) making a very large

covariance matrix with few meaningful components. Friston [15] used a method called recursive PCA to limit the number of eigenvectors describing the data. While fMRI time series usually have 20 to 60 times the length of the corresponding PET time series, there still are many more voxels than time points. False Discovery Rate (FDR) methods would be used now ([3], [4], [5]) to identify the functionally correlated voxels with a controlled expected false positive rate. The advantage of this approach is that it is an

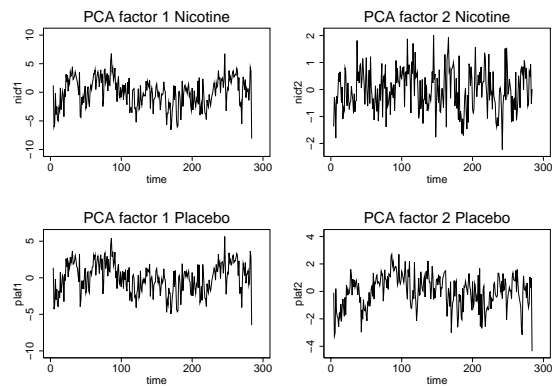


Figure 4. Graph of the two major eigenvectors of two separate PCA analyses on the same 50 activated voxels in subjects on a nicotine patch 2 hours after their last cigarette and the same subjects on a placebo patch 2 hours after their last cigarette.

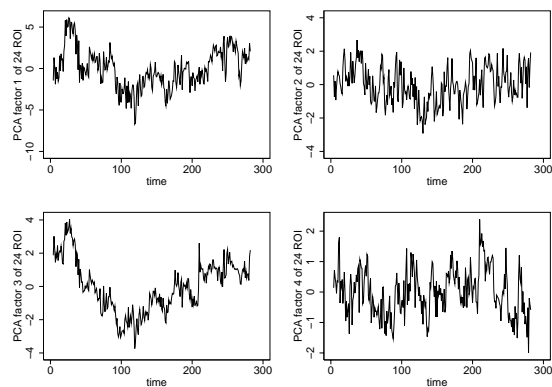


Figure 5. Graph of the 4 main eigenvectors of a PCA analysis of the mean responses of 24 active ROI. The basic task pattern is similar in all the eigenvectors, but the nonlinear trend differs among the eigenvectors, corresponding to different nonlinear trends in some of the ROI.

The advantage of this approach is that it is an

exploratory method to look for relationships; the disadvantage is that it is difficult to deal with the dimensionality of the problem on a voxel-wise basis.

Another approach to identifying components of the neural networks, especially in the case where there is no underlying task present or when the task is very complex and hard to differentiate from noise is termed Independent Component Analysis (ICA) ([26], [27]) which is discussed in several of the other recent papers.

4. Factor analysis

Factor analysis is related to PCA; however, while the aim in PCA is primarily to reduce the dimensionality of a single sample of the data, the aim in factor analysis is primarily model driven. Factor analysis assumes there is a set of underlying or “latent variables” that are related to the observed data through

$$Y = F \Lambda + E,$$

where Y without loss of generality is the standardized observed data with columns centered about their mean and divided by their standard deviation, Λ is a matrix of common coefficients or factor loadings, F is the set of specific factors assumed to have mean zero and variance one while E is the portion of Y not due to the common factors F .

In the case where F is a well specified model, the process is called confirmatory factor analysis. In the case where F is less well known, the process is called exploratory factor analysis. Exploratory factor analysis was used in Lawrence et al. [13] to explore the relationship of 29 ROIs that showed a difference in activation between a control task and an RVIP task. The difference in mean activation level between the task and the control for each of the regions and the RVIP task performance were used in an exploratory factor analysis based on the data from 25 volunteers. Three factors were identified; the first factor was composed of 15 ROIs that had a positive loading on the

response to the RVIP task, the second factor was composed of 9 ROIs that had a negative loading on the response to the RVIP task and the third factor included ROIs that did not load on the response to the RVIP task. The third factor consisted of mainly cerebellar regions. The authors used the factor analysis to conclude that there were two networks – one which supported sustained attention and one which either was not needed for sustained attention and had resources that could be used by the first network or which by being suppressed prevented distractions or attention interference.

Typically reporting the results of exploratory factor analysis involves subjectively identifying or characterizing the factors. For this reason, confirmatory factor analysis with a well-defined, *a priori* model is often preferred to exploratory factor analysis. The next section, which discusses structural equation modeling, will present an example where the *a priori* model is determined by anatomic and lesion studies.

5. Structural Equation Modeling

In fMRI, the question of whether two or more regions of the brain are associated with each other is important. For fMRI connectivity is defined in terms of the time series observed in the individual voxels or in a summary statistic, such as the mean, applied to the ROI.

Functional connectivity is the temporal correlations between spatially remote neurophysiological events [15]. It is a statement about the observed correlations, often with the task. Effective connectivity is influence one neuronal system exerts over another [16]. Effective connectivity analysis allows testing of whether a neural network is modulated by changes in other networks or changes in the experimental conditions (such as doses of a drug). 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 understand 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 ([17],[18]) first used SEM to study the connections in the rat visual cortex. In later work, Honey [19], used SEM to examine the effects of verbal working memory load on inter-cortical connectivity. Buchel and Friston [20], 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 non-linear terms in the SEM model. Coull and Buchel [21] extended this idea to the modulation of connectivity by external factors, namely, the usage of different doses of clonidine, a D2 agonist.

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 (bi-directional 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 [22]. Split-half and, more recently, jackknife methods are used to validate the results across participants [23]. The data for an SEM analysis is derived from the fMRI time series after motion correction and de-trending.

The model for the path coefficients is based on animal studies and human lesion studies.

For example, we can determine effective connectivity changes in regions activated by the RVIP task [12] with real and placebo nicotine patches two hours 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 [24] 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. Functional ROIs were defined in standard stereotaxic space by regions differentially activated in the non-smokers [13]. 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 [25]. Statistical comparisons of the modulation of the individual

connections by acute nicotine deprivation were made using Wald tests -- t-tests using the difference in the path coefficients divided by the standard error of the difference. Most notable are (1) the strengthening of the path coefficients between the SMA and the anterior cingulate and between the right posterior parietal to the visual cortex which might suggest increases in attention to external processes when on the placebo and (2) the weakening of the path coefficients between the prefrontal cortex and the posterior

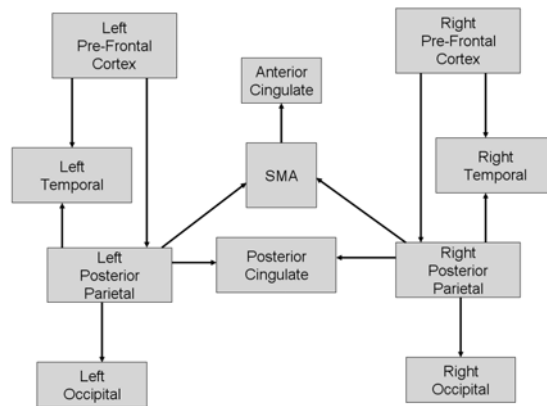


Figure 6. Expected connection pattern of the regions activated by the RVIP sustained attention and working memory task. The diagram is based on animal studies and human lesion studies.

parietal region and between the posterior parietal and the hippocampus which might suggest difficulties with goal directed tasks and attention while on placebo (acute

<u>A. Regions that decrease connectivity</u>			<u>Nicotine</u>	<u>Placebo</u>
L. Post. Parietal	to	Hippocampus	0.15	0.06*
Prefrontal	to	L. Post. Parietal	0.36	0.24**
Prefrontal	to	R. Post. Parietal	0.36	0.23**
<u>B. Regions that increase connectivity</u>				
L. Post. Parietal	to	Temporal	0.16	0.29**
SMA	to	Ant. Cingulate	0.12	0.25**
R. Post. Parietal	to	SMA	0.13	0.22*
R. Post. Parietal	to	Visual	0.15	0.22*

*** P < 0.05, ** P < 0.01**

Table 1. Path coefficients for selected connections in the RVIP task. The path coefficients were estimated separately for the nicotine patch and placebo patch conditions. Comparisons between conditions used a paired t-test.

nicotine deprivation). Interpretation of the meaning of changes in path coefficients (as in this example) is somewhat subjective and depends very much on the anatomical and lesion studies that were used to originally define the model as well as corresponding behavioral outcomes.

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.

5. Conclusion

Regression analysis with multiple comparisons corrections allows the determination of activated voxels which can then be grouped into ROI. Principal components analysis 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. Structural equation modeling 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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