Modeling Intra-Subject Correlation Among Repeated Scans in Positron Emission Tomography (PET) Neuroimaging Data

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Abstract: Many in vivo positron emission tomography (PET) neuroimaging studies record correlates of regional cerebral blood flow (rCBF) in a series of scans for each individual, usually under different experimental conditions. Typical methods for statistical analysis involve fitting voxel-specific general linear models (GLM) that assume spherical normal errors, implying that all voxel-specific rCBF measurements are independent and arise from identical normal probability distributions. While the spherical GLM provides a unified and computationally efficient approach to estimation, the likely correlations among an individual's repeated scans and heteroscedasticity between conditions prompt the use of extended statistical methodology. We outline a more general method to analyze PET data using random effects and correlated errors to model unequal variances across conditions as well as covariances (correlations) among the repeated scans for each individual. We introduce correlation maps to display intra-subject correlations between an individual's rCBF measurements from different scans. We illustrate the application of our model using data from a study of social anxiety and highlight analytical advantages over the spherical GLM. *Hum. Brain Mapping* 20:59–70, 2003. © 2003 Wiley-Liss, Inc.

Key words: repeated measures; covariance model; correlation maps; non-sphericity; mixed effects model; missing data; restricted maximum likelihood; social anxiety disorder

INTRODUCTION

Functional neuroimaging with in vivo positron emission tomography (PET) seeks to define behavior-related changes in distributed neural processing by localizing hemodynamic correlates of regional cerebral blood flow (rCBF). The typical method for statistical analysis of PET data applies voxel-spe-

cific general linear models (GLM) for rCBF that assume uncorrelated and identically distributed (spherical) normal errors [Friston, 1997], hence independence and homoscedasticity across all scans. Most PET studies record multiple scans (repeated measures) on each individual. The spherical GLM is clearly not ideal since the repeated scans for a given subject are generally not independent and may exhibit unequal variability across the different experimental conditions.

Recent extensions for functional magnetic resonance imaging (fMRI) extend the GLM by including covariance models that address temporal correlation among serial brain activity measurements. Bullmore et al. [1996], for example, propose a first-order autoregressive (AR(1)) covariance model, which is extendable to a p -order autoregressive model. Purdon et al. [2001] apply a covariance model proposed by Diggle et al. [1994] for the statistical analysis of longitudinal data, which decomposes the temporal correla-

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tion into an AR(1) model plus white noise. Friston et al. [1995] and Worsley and Friston [1995] augment the GLM by inducing a known serial correlation structure using temporal smoothing with a Gaussian kernel. The temporal resolution in PET generally provides little justification for use of an autoregressive serial covariance model, so these fMRI statistical methods have limited applicability in our setting. However, we demonstrate that using alternative covariance models for PET data that reflect correlations among the repeated scans on each individual has analytical advantages.

Random effects analyses of *in vivo* neuroimaging data specify a two-stage (or higher) model and implicitly assume very basic parametric structures on intra-subject correlations such as compound symmetry or equicorrelation. Several authors, including Friston et al. [1999], Holmes and Friston [1998], Penny [2001], and Worsley et al. [2002] comment on the advantages of conducting random or mixed effects analyses relative to fixed effects models. For PET, fixed effects models assume that changes in brain activity associated with a particular behavior (experimental condition) are equal for all subjects and differ only due to random variability in rCBF. In contrast, mixed effects models include this constant activity across all subjects, plus an effect expressing random subject-to-subject variability, possibly related to a specific condition, plus random error. With appropriate random sampling and correct specification of a fixed effects model, estimates of fixed effects pertain to population parameters from which the sample was selected. A problem in multi-subject neuroimaging studies is that correctly specifying a model requires accounting for repeated measures, typically accomplished by including a fixed subject effect, so resulting inferences apply only to subjects who participate in the study. Modeling random subject effects, on the other hand, permits inferences to extend to the population represented by the study sample. Beyond generalizing inferences to a population, random effects contribute to an overall within-subject covariance structure representing, for example, correlation among repeated scans on each individual.

Current applications of random effects models to neuroimaging data are relatively simple and perform multi-stage parameter estimation. For example, Holmes and Friston [1998] describe a procedure that fits subject-specific models at the first stage, and the second stage performs between-subject estimation by fitting a model to the subjects' parameter estimates from the first stage. It is important to note that the second stage model specifies subject-specific parameters as the response variable rather than estimates of these parameters. Thus, using parameter estimates as "raw" data introduces random variability in the second stage. Also, the covariance matrix of these estimates depends on the number of measurements and possibly the acquisition times, and the second stage analysis does not take this into account [Verbeke and Molenberghs, 2000]. Iterative estimation procedures are standard in the statistical literature for random or mixed effects models. These methods simultaneously estimate fixed effects and covariance parameters. The algorithms permit flexible model specification and accommodate

missing or unbalanced data. The drawback of simultaneous parameter estimation in neuroimaging data analyses is that the computational burden is much greater than two-stage estimation.

The non-independence and heteroscedasticity among an individual's repeated scans prompt the use of extended statistical methodology. We propose a novel application of mixed effects models to incorporate unequal variances across experimental conditions as well as covariances, hence correlations, among a subject's brain activity measurements from repeated scans. This approach extends the GLM by relaxing the independent and identically distributed errors (scans) assumptions and extends random effects models applied to *in vivo* neuroimaging data by allowing estimation of a range of parametric covariance structures. We combine the theoretical two-stage random effects model construct into a single stage for estimation and use restricted maximum likelihood (REML) to simultaneously estimate fixed effects and covariance parameters. Despite the computational demand of performing REML estimation for mixed models, we illustrate its feasibility in PET data analyses. We introduce the use of correlation maps to display intra-subject correlations between rCBF measurements from different experimental conditions. We highlight analytical advantages of our proposed model over standard methods, including increased precision of the estimators, increased statistical power, and the ability to accommodate missing data.

The motivating study for this article is a PET analysis that investigates neural processing underlying social anxiety related to generalized social anxiety disorder, also called social phobia (SP). We formulate our model in the context of this study, but the general methodology also pertains to PET analyses with different study designs. After briefly describing the rationale and design of the SP study, we provide an exposition of the mixed effects model, comment on specific covariance models that address various sources of design-based correlation and random variation, and illustrate applicability of our model using PET data from the SP study.

DESCRIPTION OF EXPERIMENTAL DATA

SP is a prevalent, debilitating, and neglected disorder characterized by fear and avoidance of social situations that involve observation or evaluation by others. The neurobiology of social anxiety disorder remains poorly understood and the specific brain responses involved in successful treatment are currently unknown. Our SP study includes twelve subjects who met criteria for a clinical diagnosis of social anxiety disorder. The first scientific objective is to define alterations in distributed neural processing associated with SP and related avoidant symptoms by comparing brain activity for different experimental conditions. The study consists of conditions that evoke social and generic anxiety as well as two control states. A second goal of this study is to identify task-specific changes in distributed neural processing related to use of an indicated pharmacological intervention, nefazodone.

For each subject, we collected eight single-frame PET images using the blood flow tracer $[^{15}\text{O}]\text{H}_2$ in each of two study periods, before and after pharmacological treatment for SP, for a total of 16 scans. Two replicates of four study conditions (stimuli) comprise the eight scans within each period. The study conditions include rest (eyes closed, ears unoccluded), an imagery control task in which subjects mentally reenacted a nonsocial scene (e.g., a serene beach or forest), a social anxiety inducing imagery task in which subjects mentally reenacted a personal social situation associated with fear and avoidance (e.g., public speaking), and lastly a generic anxiogenic control task in which subjects were probed to respond quickly to challenging mental arithmetic questions (e.g., counting backward from 3,962 by 13).

THE STATISTICAL MODEL

We begin by presenting a general form of the statistical model for hemodynamically estimated brain activity. For a given subject, let the index $s = 1, \dots, S$ represent the scan number, and each scan reflects three components (p, r, c) denoting the period $p = 1, \dots, P$ (e.g., treatment period), replicate or session $r = 1, \dots, R$, and condition or stimulus $c = 1, \dots, C$. For the SP study, we define the indices as $p = 1$ (pre-treatment) or 2 (post-treatment) and $c = 1$ (rest), 2 (imagery control), 3 (social anxiety), or 4 (math). The total number of scans for each individual is $S = \text{PRC}$. The design of the SP study includes $S = 16$ scans for each subject with $P = 2, R = 2$, and $C = 4$. The mixed effects model for the k th subject's regional brain activity at location (voxel) v is

$$\mathbf{Y}_k(v) = \mathbf{X}_k \boldsymbol{\beta}(v) + \mathbf{Z}_k \mathbf{d}_k(v) + \boldsymbol{\epsilon}_k(v), \quad (1)$$

where $\mathbf{Y}_k(v) (S \times 1)$ is a vector of rCBF measurements for the k th subject at location v , $\mathbf{X}_k (S \times q)$ is the fixed effects design matrix with columns specifying conditions, sessions, periods, and covariates, and $\boldsymbol{\beta}(v) (q \times 1)$ is the unknown fixed effects parameter vector at voxel v relating design variables and covariates to the response. The mixed effects model (1) extends the GLM by incorporating random subject-specific fluctuations in brain activity about the overall (population) mean. The matrix $\mathbf{Z}_k (S \times m)$ is the random effects design matrix, $\mathbf{d}_k(v) (m \times 1)$ is a subject-specific vector of random effects parameters for voxel v , and $\boldsymbol{\epsilon}_k(v)$ is random error in the k th subject's brain activity about his/her individualized mean. We assume that brain activity measurements from different subjects are independent and

$$\begin{bmatrix} \mathbf{d}_k(v) \\ \boldsymbol{\epsilon}_k(v) \end{bmatrix} \sim N \left(\begin{bmatrix} \mathbf{0} \\ \mathbf{0} \end{bmatrix}, \begin{bmatrix} \boldsymbol{\Delta}_v & \mathbf{0} \\ \mathbf{0} & \sigma_v^2 \mathbf{V}_{kv} \end{bmatrix} \right). \quad (2)$$

The random components $\mathbf{d}_k(v)$ and $\boldsymbol{\epsilon}_k(v)$ are independent. The corresponding covariance matrices $\boldsymbol{\Delta}_v$ and $\sigma_v^2 \mathbf{V}_{kv}$ are unknown and unstructured in general, but we may apply parametric covariance patterns in a particular application.

Mean model

The mean model from equation (1), along with the corresponding assumptions, is

$$E(\mathbf{Y}_k(v)) = \mathbf{X}_k \boldsymbol{\beta}(v). \quad (3)$$

All voxels include the same design variables, but the parameter vector at each voxel is distinct. The mean structure is analogous to that of a GLM [Friston et al., 1990], so standard model specification and variable adjustment techniques apply. The mean model for the SP data includes separate pre- and post-treatment condition effects and covariate adjustments for global cerebral blood flow (gCBF), X_{gCBF} . Let $\mathbf{X}_{ks} = (X_{ks11}, \dots, X_{ks14}, X_{ks21}, \dots, X_{ks24}, X_{\text{gCBF}})$ represent a row-vector from $\mathbf{X}_k$, identifying the design settings for scan s , where X_{kspc} equals one if scan s from subject k corresponds to period p and condition c , and X_{kspc} equals zero otherwise. Our model describes the k th subject's mean brain activity for scan s at location v by

$$E(Y_{ks}(v)) = \mathbf{X}_{ks} \boldsymbol{\beta}(v) = \sum_{p=1}^4 \sum_{c=1}^4 X_{kspc} \beta_{pc}(v) + \beta_0(v) X_{\text{gCBF}}, \quad (4)$$

where $\boldsymbol{\beta}(v) = (\beta_{11}(v), \dots, \beta_{14}(v), \beta_{21}(v), \dots, \beta_{24}(v), \beta_0(v))'$. The model distinguishes pre- and post-treatment mean parameters.

Covariance model

Including random effects generalizes results beyond the specific factor levels included in a study. We select variables in $\mathbf{Z}_k$ as a subset of those in $\mathbf{X}_k$, so all random effects represent subject-level deviations from an associated overall fixed component. We separate rCBF into the sum of a stimulus-specific component that is constant across all individuals (population effect), an effect that varies randomly for each subject based on experimental design features and individual characteristics, and within-subject random variation. As a practical example, consider a study of brain activity alterations evoked by the stress associated with immersing the right hand in a bucket of 38°F water for 20 seconds. Our model regards an individual's neural responses associated with this stressor as the sum of a constant (mean) portion common to all individuals, plus a random increment (decrement) based on inter-subject variability due, for example, to each individual's personal stress or pain tolerance, plus within-subject random error.

Inclusion of random effects $\mathbf{d}_k(v)$ leads to the covariance structure

$$\boldsymbol{\Sigma}_k(v) = \text{Var}(\mathbf{Y}_k(v)) = \mathbf{Z}_k \boldsymbol{\Delta}_v \mathbf{Z}_k' + \sigma_v^2 \mathbf{V}_{kv}. \quad (5)$$

The component $\mathbf{Z}_k \boldsymbol{\Delta}_v \mathbf{Z}_k'$ introduces variation due to individualized random fluctuations about the population mean,

and $\sigma_v^2 \mathbf{V}_{kv}$ incorporates residual variance associated with the measurement of behavior-related brain activity. Specific parametric structures are applicable to Δ_v and $\mathbf{V}_{kv}$. For example, the class of general linear covariance models is defined by $(\Sigma_k(v))_{ij} = \sum_{g=1}^G \eta_{vg} \mathbf{A}_{ij}$, where η_{vg} is an unknown covariance parameter, $\mathbf{A}_{ij}$ is known, and the resulting covariance matrix is positive definite. This class contains compound symmetry $\Sigma_k(v) = \sigma_v^2 \mathbf{I}_S + \sigma_{v1} \mathbf{J}_S$, where $\mathbf{I}_S$ is the $(S \times S)$ identity matrix and $\mathbf{J}_S$ is the $(S \times S)$ unity matrix, and numerous other parametric structures that may apply to PET data. Defining the (i, j) th element of the covariance matrix as $(\Sigma_k(v))_{ij} = \sigma_v^2 \rho_v^{|i-j|}$, $\rho_v > 0$, specifies an autoregressive structure that is often appropriate to model autocorrelation in fMRI studies and some PET studies in which the correlation between two scans is a decreasing function of the acquisition time lag separating the scans.

For the SP study, we model correlation in task-related brain activity among all sixteen scans for each individual. We relax sphericity assumptions of the GLM by using a parametric structure for the covariance model that reflects the study conditions and experimental design. Due to varying manifestations and severity of SP symptoms across individuals, our mean model specifies an overall activation level associated with each condition in the pre- and post-treatment periods, and the individualized random effects incorporate deviations for each condition and study period. The random effects for each subject covary among the four conditions and independently covary among the pre- and post-treatment periods.

The random effects induce covariances between an individual's rCBF measurements from repeated scans, under the various study conditions and periods. Let

$$\Delta_v = \begin{bmatrix} \Gamma_v & \mathbf{0} \\ \mathbf{0} & \Phi_v \end{bmatrix},$$

where $\Gamma_v(4 \times 4)$ contains elements $(\gamma_{vcc'})$ corresponding to the four study conditions, c and $c' = 1, \dots, 4$ and $\Phi_v(2 \times 2)$ has elements $(\phi_{vpp'})$ corresponding to pre- and post-treatment periods, p and $p' = 1, 2$. Two rCBF measurements on an individual from conditions and periods (c, p) and (c', p') have covariance $\gamma_{vcc'} + \phi_{vpp'}$, and the variance of $Y_{kpc}(v)$ is $\sigma_v^2 + \gamma_{vcc} + \phi_{vpp}$. Our model extends the sphericity assumptions of the GLM, since the variances differ across conditions and treatment periods, and we do not restrict covariances to zero. The correlation between two rCBF measurements follows directly from the structured variances and covariances and in the SP study is given by

$$\rho(Y_{kpc}(v), Y_{kp'c'}(v)) = \frac{\gamma_{vcc'} + \phi_{vpp'}}{\sqrt{(\sigma_v^2 + \gamma_{vcc} + \phi_{vpp})(\sigma_v^2 + \gamma_{v'c'} + \phi_{v'p'})}}.$$

We omit the index r since variances and covariances do not depend on the replicate number. We construct correlation maps for the SP study that permit visual examination of within-subject correlations between rCBF measurements

across conditions and periods. The full covariance matrix at voxel v for each individual in the SP study is

$$\Sigma_k(v) = (\mathbf{J}_4 \otimes \Gamma_v) + (\Phi_v \otimes \mathbf{J}_8) + \sigma_v^2 \mathbf{I},$$

where $\otimes$ is the Kronecker product. The first term contains variances and covariances for the study conditions, the second summand includes variances and the covariance for the treatment periods, and the final term reflects independent random variation.

Missing data

The mixed effects model outlined above can accommodate missing data (scans) in contrast to other methods requiring complete data. As is common of PET and fMRI studies, the SP study contains missing data for several individuals. One subject completed the pre-treatment study period, but did not participate in post-treatment scanning. Another subject completed all scanning sessions, except for the post-treatment anxiogenic control condition (math task). Three additional individuals do not have data for the math task during the pre-treatment period. We include these subjects' partially observed data in our analysis assuming that the probability of not recording a response (rCBF) does not depend on the unobserved level of brain activity. This assumption corresponds to ignorably missing data, for which our model provides valid inferences [Little and Rubin, 2002].

Nonignorably missing data arise if, for example, brain activity exceeds the scanner's detection limit. The assumption of ignorable missingness is often tenable in neuroimaging applications, since common causes for missing data are design changes, loss to follow up, scanner malfunction, head movement, and susceptibility artifacts. Implementing simple random effects models currently applied in neuroimaging [Holmes and Friston, 1998] and traditional multivariate linear models requires complete balanced data. A major advantage of the mixed effects model outlined here is its ability to handle ignorably missing data.

Estimation and testing

The standard fixed effects estimator due to Laird and Ware [1982] is

$$\hat{\beta}(v) = \left(\sum_{k=1}^n \mathbf{X}_k' \hat{\Sigma}_k^{-1}(v) \mathbf{X}_k \right)^{-1} \sum_{k=1}^n \mathbf{X}_k' \hat{\Sigma}_k^{-1}(v) \mathbf{Y}_k(v), \quad (6)$$

where estimates of the parameters in $\Sigma_k(v)$, say $\eta(v)$, are obtainable using REML. The REML estimator $\hat{\eta}(v)$ maximizes the likelihood function of the ordinary least-squares (OLS) residuals from a GLM, after replacing $\beta(v)$ with (6), based on the marginal distribution of $\mathbf{Y}_k(v)$. We implement REML estimation instead of maximum likelihood (ML) because ML is known to produce biased estimators of variance components. In practice, iterative algorithms such as Newton-Raphson and Expectation-Maximization (EM) are con-

venient tools for simultaneously estimating $\beta(v)$ and $\eta(v)$. Further details are available in Lindstrom and Bates [1988].

Conditional on $\eta(v)$, $\hat{\beta}(v)$ follows a multivariate normal distribution with mean β and covariance matrix $(\sum_{k=1}^n \mathbf{X}_k' \Sigma_k^{-1}(v) \mathbf{X}_k)^{-1}$. Therefore, hypothesis testing for parameters $\theta(v) = \mathbf{C}\beta(v)$ evolves as a straightforward extension to the GLM. We test $H_0: \theta(v) = \theta_0$ versus $H_a: \theta(v) \neq \theta_0$ (or $\theta(v) > \theta_0$), for specified θ_0 , using

$$F = (\text{rank}(\mathbf{C}))^{-1} (\hat{\theta}(v) - \theta_0)' \times \left[\mathbf{C} \left(\sum_{k=1}^n \mathbf{X}_k' \hat{\Sigma}_k^{-1}(v) \mathbf{X}_k \right)^{-1} \mathbf{C}' \right]^{-1} (\hat{\theta}(v) - \theta_0),$$

which under the null hypothesis approximately follows an F-distribution with $\text{rank}(\mathbf{C})$ numerator degrees of freedom and ν denominator degrees of freedom. Various methods exist for estimating ν from the data, and these approximations typically yield similar P values and statistical inferences for repeated measures studies [Verbeke and Molenberghs, 2000]. We use $\nu = N - \text{rank}([\mathbf{X} \ \mathbf{Z}])$, with $\mathbf{X} = \text{stack}(\mathbf{X}_1, \dots, \mathbf{X}_n)$, where the stack function vertically concatenates its arguments, and $\mathbf{Z} = \text{diag}(\mathbf{Z}_1, \dots, \mathbf{Z}_n)$ is block diagonal. Approximate noncentral distributions are also available for F under H_a and are useful for computing statistical power. Use of F helps resolve the downward bias of alternative Wald test statistics [Dempster et al., 1981]. When $\mathbf{C}$ has only one row, the square root of F is equivalent to an approximate t -statistic with ν denominator degrees of freedom, reducing F-statistic activation maps to equivalent t -maps.

RESULTS: STUDY OF SOCIAL ANXIETY DISORDER

We estimate mean rCBF for the four study conditions preceding and following the 12-week nefazodone pharmacotherapy using our mixed effects model. All study participants met criteria for a clinical diagnosis of social anxiety disorder and experienced significant levels of occupational, family, or personal disability related to social anxiety. As a disorder characterized by fear and avoidance of being observed or evaluated, many of the subjects experienced anxiety while lying in the PET scanner, even during the rest condition. To mitigate the potential confounding of “baseline” anxiety, we include the initial rest condition as an effect in our mean model and estimate behavior-related changes in brain activity for the other study conditions relative to estimated activity during the initial rest scan.

Mean effects

Denote the rCBF measure of brain activity $Y_{ks}(v)$ explicitly by $Y_{kprc}(v)$, when scan s is obtained in period p , replicate r , and condition c . We estimate adjusted means

$$E[Y_{kprc}^*(v)] = E[Y_{kprc}(v)] - E\left[\frac{Y_{k111}(v) + Y_{k121}(v)}{2}\right] = E[Y_{kpc}(v) - Y_{k1 \cdot 1}(v)], \quad (7)$$

where the dot notation means that we average over the missing index (r here). Note that the adjustment equates to estimating means of differences $Y_{kprc}(v) - Y_{k1 \cdot 1}(v)$, although we do not actually perform these data transformations. For each voxel, we estimate the impact of pharmacotherapy for condition c , say $\tau_c(v)$, in a classical sense using

$$\hat{\tau}_c(v) = \hat{E}(Y_{kprc}^*(v)|p = 1) - \hat{E}(Y_{kprc}^*(v)|p = 2), \quad (8)$$

where we are primarily interested in identifying large departures from zero for $c = 3$ (social anxiety mental imagery) and $c = 1$ (rest). Since we apply the same adjustment to each subject’s pre- and post-treatment brain activity estimates, the estimate of treatment impact is equivalent to $\hat{E}(Y_{kprc}(v)|p = 1) - \hat{E}(Y_{kprc}(v)|p = 2)$.

Figure 1 presents results from our mixed effects model, displaying cortical areas with significantly higher activation during the social anxiety provocation condition relative to the control mental imagery condition prior to pharmacotherapy. The significant voxels that appear in all activation maps have corresponding P values < 0.001 , uncorrected for multiple comparisons [Worsley et al., 1996]. These axial slices in Figure 1 range from +1 mm to +8 mm. The significant frontal cortical activation spans the precentral and inferior frontal gyri and suggests that these areas play a fundamental role in the neural processing underlying social anxiety among individuals with SP.

Figures 2 and 3 illustrate the impact of medication on the neural correlates of social anxiety. Figure 2 shows cortical areas that exhibit a significant reduction in activity related to social anxiety provocation following pharmacotherapy (relative to the pre-medication social anxiety condition). Interestingly, it appears that effective pharmacotherapy is associated with diminished activation of this social anxiety-related frontal cortical region (Fig. 2), supporting its role in neural processing associated with social anxiety. Since all subjects in this study met criteria for a clinical diagnosis of SP, the subjects predictably report experiencing moderate anxiety during the pre-treatment rest condition, probably related to the sense of being observed or evaluated while positioned in the scanner. Therefore, we compare the pre- vs. post-treatment rest conditions to explore changes in distributed neural processing related to use of the medication nefazodone. Figure 3 shows that prior to pharmacotherapy, the anxiety-related brain regions exhibit significantly increased neural activity at rest relative to resting rCBF levels observed during the post-treatment period.

Correlation maps

We construct correlation maps that present mixed model-based estimates of correlations between rCBF measurements for all pairs of experimental conditions. These maps further

Figure 1.

Activation maps for social anxiety condition vs. the imagery control condition during the pre-treatment period. $P < 0.001$ (uncorrected) at each voxel. The axial slices range from +1 to +8 mm.

Figure 2.

Activation maps for pre-treatment social anxiety condition vs. post-treatment social anxiety condition. $P < 0.001$ (uncorrected) at each voxel. The axial slices range from +8 to +15 mm.

Figure 3.

Activation maps for pre-treatment rest condition vs. post-treatment rest condition. $P < 0.001$ (uncorrected) at each voxel. The axial slices range from +8 to +15 mm.

Figure 4.

Model-based maps of voxel-by-voxel correlations of an individual's rCBF measurements for various pairs of study conditions, within and between treatment periods.

support use of our mixed effects model, since under GLM analyses, they would appear as monochrome (blue) maps reflecting zero correlations. The model-based correlation maps display distributed patterns of similarity in localized brain activity between the study conditions. We estimate an individual's correlation matrix from his/her estimated covariance matrix $\hat{\Sigma}_k(v)$ using

$$\hat{\mathbf{R}}_k(v) = \hat{\mathbf{D}}_k^{-1/2}(v) \hat{\Sigma}_k(v) \hat{\mathbf{D}}_k^{-1/2}(v), \quad (9)$$

where $\hat{\mathbf{D}}_k(v)$ is a diagonal matrix with diagonal elements matching those of $\hat{\Sigma}_k(v)$. For the PET SP study, the full covariance matrix for an individual's rCBF measurements is a 16×16 matrix corresponding to the two treatment periods, two replicates, and four conditions. Mapping the correlation at each voxel, we present a condensed 8×8 array since our model specifies $\text{Corr}(Y_{prc}(v), Y_{p'r'c'}(v)) = \text{Corr}(Y_{prc}(v), Y_{p'r'c'}(v))$, where $c \neq c', r \neq r'$, and either $p = p'$ or $p \neq p'$, i.e., correlations between rCBF measurements in different conditions or periods do not depend on the replicates in which they are obtained.

The correlation maps appear in Figure 4 for the SP study. The maps along the main diagonal represent model-based estimates of correlations between the two replicates of a study condition within the same treatment period. For instance, the map of the (anxiety, anxiety) combination in the pre-treatment period, presents distributed correlations of neural activity between the first and second social anxiety imagery scans within the period. Off-diagonal maps display correlation estimates between rCBF measurements in the conditions and periods corresponding to the aligned rows and columns, irrespective of replicate.

The correlation maps reveal relatively high correlations, particularly along the midline, spanning regions including the medial frontal cortex, anterior and posterior cingulate, and precuneus. rCBF measurements within the same period generally exhibit high correlations, and smaller correlations exist between rCBF from conditions in different periods. The strongest correlations appear in the post-treatment period between rCBF measurements obtained in replicates of the same condition, particularly in the frontal and visual cortices. Within both treatment periods, high correlations are

prominent among the four conditions in the cingulate cortices and bilaterally in the extrastriate cortex. There are also fairly high rCBF correlations in the lateral frontal cortex between all study conditions during the post-treatment period. The correlation maps identify the extent to which specific voxels exhibit similar activity for pairs of study conditions and periods, but do not measure the magnitudes of activity for these pairs. Therefore, correlation maps provide a useful supplement to activation maps.

Comparison with the general linear model

A typical neuro-activation statistical analysis of in vivo PET data applies a GLM with spherical normal errors to each voxel, implying independence and homoscedasticity across all scans, and estimates parameters using OLS. Employing the SP PET data, we perform a comparative analysis using the spherical GLM and the described mixed effects model that incorporates heteroscedasticity and covariances among each individual's repeated scans. Figure 5 shows significant task-related activations from both the repeated scans covariance model and the GLM for a contrast of the post-treatment math (provocative mental arithmetic) condition vs. the post-treatment rest condition. Figure 5 supports the contention that the repeated scans covariance model offers more powerful localized tests of the contrast, detecting more task-related activations than the GLM with equal probabilities of type-I errors. The repeated scans covariance model detects significant math task-related activations in the anterior cingulate and middle frontal cortices, putamen, insula, and caudate nucleus, all of which are undetected by the GLM. Both statistical models identify right superior temporal gyral activation, but the spatial extent of this activation for the mixed effects model clearly exceeds that of the spherical GLM.

Analytically, the mixed effects model and the GLM produce unbiased estimators of fixed effects parameters and associated contrasts, assuming known variances and covariances. However, the mixed effects model reduces variability about the fixed effects estimators, since the difference between the covariance matrices of these estimators $V(\hat{\beta}_{OLS}) - V(\hat{\beta}_{RS})$ is a positive semidefinite matrix (RS represents the use of a repeated scans covariance model). Empirically, the mixed effects model reduces variability of the fixed effects estimates by borrowing strength from repeated scans on each individual, yielding the observed increase in statistical power.

DISCUSSION

Use of a mixed effects model to analyze PET data improves existing methods of image analysis by providing a flexible, unified framework for estimation and inference that allows a variety of covariance structures to account for correlation among each individual's repeated scans. In addition, the mixed effects methodology pools strength among an individual's repeated scans, which capitalizes on the intra-subject correlation to increase statistical power. In-

creased power is particularly advantageous in studies that attempt to detect subtle changes in localized task-related brain activity, since most in vivo neuroimaging studies enroll a small to moderate number of subjects. Distributional assumptions underlying the mixed effects model match characteristics of most PET data far better than the sphericity assumptions of the GLM. In our data example, we fit a relatively parsimonious covariance model that reflects important aspects of the study design that are disregarded by the GLM.

Model-based correlation maps are extremely useful descriptive tools for illustrating associations between rCBF from different scans. Such maps reveal voxel-specific correlations in brain activity rather than quantifying behavior-related changes in activation, thus they serve as useful supplements to standard activation maps. Although we utilize a parsimonious covariance model, the correlation maps suggest that a further simplified compound symmetric model is inappropriate for the data. Compound symmetry assumes equal variances $\sigma_v^2 + \sigma_{v1}$ for all repeated measures and equal covariances σ_{v1} between all pairs of scans and results from fitting a single random effect (intercept) for each individual. Currently, most two-stage random effects models applied to neuroimaging data implicitly formulate compound symmetric covariance models. Our within-treatment period correlation maps differ markedly from the between-treatment maps, indicating the inadequacy of a compound symmetric model and further supporting use of the more general framework that we outline to model covariance matrices.

GLMs with uncorrelated errors produce monochrome zero-correlation maps. The mixed model estimator $\hat{\beta}(v)$ and its variability deviate from corresponding GLM-based quantities to an extent determined by the difference between the weight matrix $\hat{\Sigma}_k^{-1}(v)$ and the identity matrix. This difference is revealed by contrasting our correlation maps (Fig. 4) with monochrome maps, again demonstrating the importance of modeling within-subject covariance. The estimator $\hat{\beta}(v)$ is generally more efficient than the OLS estimator from a GLM when the marginal distribution of rCBF specified by the mixed model is correct.

Another important advantage of the mixed effects model in the context of in vivo neuroimaging is its ability to accommodate missing data. Missing data often arise in practice, and others have noted the need for statistical methods that incorporate partially observed data into analyses. For example, the importance of handling missing data is evident in Friston et al.'s [2002] statement concerning Bayesian statistical models for neuroimaging data "...the real power of Bayesian inference lies in coping with incomplete data or unbalanced designs. ..." Discarding missing data loses information, thus decreases statistical power of tests and efficiency of estimators, and risks introducing bias into a statistical analysis. Provided that the data are ignorably missing, the model that we outline avoids these shortcomings and yields asymptotically unbiased estimators for the fixed effects parameters.

Figure 5.

Comparison of activation maps for post-treatment math condition vs. post-treatment rest condition. The repeated scans covariance model detects more activations than the spherical GLM. $P < 0.001$ (uncorrected) at each voxel. The axial slices range from 0 to +20 mm.

One drawback of iterative estimation procedures is computing time since we perform estimations at each voxel [Worsley et al., 2002]. For PET data as well as fMRI data with short time series, it is feasible, although less time efficient, to implement iterative estimation techniques. We do not attempt to produce more than demonstration software for our analyses, so additional gains in computing efficiency are likely. We perform our analysis using MATLAB R. 12 and SAS v. 8.02 and generally run our models over a weekend. This computational demand required to effect a thorough statistical analysis that provides more valid inferences seems reasonable given that in vivo neuroimaging studies involve significant financial costs and are carried out over several months (or years).

The neuroimaging statistical software SPM99 uses OLS estimation for parameters in a GLM with spherical normal errors. SPM2, currently available in a beta version, relaxes assumptions of the GLM by estimating a single voxel-specific variance component and using REML estimates to whiten the data, permitting subsequent Gauss-Markov estimation of regression coefficients. One implication is that SPM2 implements fixed-effects models, so that one must emulate a random effects model by fitting its components in multiple stages. The method that we outline consolidates estimation of the multiple stages of a random effects model into a single iterative process, simultaneously estimates fixed effects and covariance parameters, and permits a variety of parametric covariance structures. Our approach addresses within-voxel correlations and heteroscedasticity. Extending our framework to incorporate spatial correlations between localized brain activity measurements remains an area of active research.

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