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Modeling Dose-Dependent Neural Processing Responses Using Mixed Effects Spline Models: with application to a PET study of ethanol

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Abstract

For functional neuroimaging studies that involve experimental stimuli measuring dose levels, e.g. of an anesthetic agent, typical statistical techniques include correlation analysis, analysis of variance or polynomial regression models. These standard approaches have limitations: correlation analysis only provides a crude estimate of the linear relationship between dose levels and brain activity; ANOVA is designed to accommodate a few specified dose levels; polynomial regression models have limited capacity to model varying patterns of association between dose levels and measured activity across the brain. These shortcomings prompt the need to develop methods that more effectively capture dose-dependent neural processing responses. We propose a class of mixed effects spline models that analyze the dose-dependent effect using either regression or smoothing splines. Our method offers flexible accommodation of different response patterns across various brain regions, controls for potential confounding factors, and accounts for subject variability in brain function. The estimates from the mixed effects spline model can be readily incorporated into secondary analyses, for instance, targeting spatial classifications of brain regions according to their modeled response profiles. The proposed spline models are also extended to incorporate interaction effects between the dose-dependent response function and other factors. We illustrate our proposed statistical methodology using data from a PET study of the effect of ethanol on brain function. A simulation study is conducted to compare the performance of the proposed mixed effects spline models and a polynomial regression model. Results show that the proposed spline models more accurately capture varying response patterns across voxels, especially at voxels with complex response shapes. Finally, the proposed spline models can be used in more general settings as a flexible modeling tool for investigating the effects of any continuous covariates on neural processing responses.

Keywords

Regression splines; Smoothing splines; Dose-dependent effect; Mixed effects spline models; Continuous covariates

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INTRODUCTION

Functional neuroimaging, including positron emission tomography (PET) and functional magnetic resonance imaging (fMRI), is invaluable for studying the effect of an experimental stimulus on human brain function measured *in vivo*. An experimental stimulus represents the administration of any substance or the performance of any task that may invoke neural processing alterations. Here, we focus on experimental stimuli corresponding to particular dose levels of an administered substance, such as ethanol or an anesthetic agent, and we obtain serial measures of dose-dependent brain function by repeatedly acquiring PET or fMRI scans. The primary research objective is to determine how the measured brain activity changes as a function of varying dose levels.

Statistical analyses of data from functional neuroimaging studies targeting dose-dependent effects often employ techniques such as correlation analysis (Calhoun et al. 2004) and ANOVA (Luo et al., 2003; White et al., 2000). Correlation analyses enable the detection of a linear association between the administered dose of a substance and the measured brain response. ANOVA offers a slightly more flexible alternative by modeling mean parameters corresponding to particular dose levels as well as by enabling subsequent testing to evaluate differences between doses. Several studies also apply polynomial regression to detect systematic changes in brain function across stimulus repetitions (Clark, 2002; Sporn, 2003; Buchel, 1996 and 1998; Van Bogaert, 1998). Polynomial regression provides an explicit parametric model that may capture specific forms of nonlinearities and is straightforward to apply in practice.

Though broadly applied, the standard statistical techniques have limitations for modeling the dose-dependent effects in neuroimaging studies. The simple correlation analysis provides only a crude measure of (linear) association between dose levels and the measured brain activity. ANOVA models are not well-suited for studies that incorporate a wide range of dose levels, since such models do not yield parsimonious models, which poses particular challenges given the small sample sizes often utilized in functional neuroimaging studies. Polynomial regression provides a convenient modeling framework for characterizing the response function but it suffers from several major limitations. First, the choice of the highest order polynomial in the model is often arbitrary. For simplicity, linear, quadratic and cubic models are common in practice. Higher order polynomial models often lead to difficulties in interpretation. Secondly, polynomials lack flexibility in the sense that the shape of a polynomial regression curve is restricted by its parametric form, which is determined by its model order, and changing the behavior of the polynomial function near one stimulus level inevitably has radical implications for its behavior for any other stimulus levels (Ramsay, 1988). Consequently, polynomial regression is not effective at capturing certain complex response patterns. Thirdly, when fitting polynomial regression models to functional neuroimaging data, a common polynomial order is generally applied to all brain areas. Since the shape of a polynomial fit is restricted by its model order, polynomial regression models with a globally specified model order may have limited capacity to detect varying response patterns across different brain regions. This is especially problematic with low order polynomial regressions, since they will likely suppress the presence of higher-order trends in some brain regions.

The heterogeneity in the regional brain activity prompts the use of a more flexible statistical method for analyzing dose-dependent effects on neural processing. We propose an approach that employs mixed effects spline models to characterize the relationships between measured brain activity and dose levels. The basic idea underlying spline modeling is to construct a dose-dependent response profile for each voxel by concatenating locally fitted line segments. Given the local nature of splines, they are capable of capturing a wide range of nonlinear shapes of

response profiles and hence are an ideal tool for modeling different neural processing patterns across the brain.

We consider two types of splines: regression splines and smoothing splines. With regression splines, one selects a sequence of knots, segmenting the observed dose range into consecutive intervals, and then fits a piecewise polynomial model within each interval. Regression splines are attractive due to the computational and statistical simplicity. However, a drawback concerning the use of regression splines is that it requires the specification of the number and locations of knots. As an alternative, smoothing splines use every distinct observed covariate value (dose level) as a knot and describe the response profile with a twice-differentiable smooth function. Smoothing splines are determined by minimizing a penalized sum of squares that consists of two parts. The first part measures the goodness-of-fit of the spline using the squared errors between the observed and fitted values, and the second part penalizes the roughness of the spline. Green and Silverman (1994) show that the smoothing spline minimizing the penalized sum of squares is a natural cubic spline. In our proposed mixed effects spline model for dose-dependent brain responses, we also incorporate a random effect to account for the subject-to-subject variability in measured brain activity. One can employ the estimates from our proposed spline models in a secondary inferential framework, such as deriving conventional t-statistic maps, and in a descriptive analysis such as clustering to identify brain regions that demonstrate similar patterns of dose-dependent neural processing responses.

For the proposed spline models, estimation for the mixed effects regression spline model is straightforward and can be conveniently carried out using standard software for linear mixed models. Fitting the smoothing spline model is more challenging. However, we show that, through model reparameterization, the proposed mixed effects smoothing spline model can also be estimated under the linear mixed model framework. Therefore, both of the proposed spline models can be estimated using standard statistical software, which facilitates their application in neuroimaging studies.

Our proposed methodology is motivated by a PET study that investigates dose-dependent effects of ethanol on human brain function. Other potential applications include mental health studies that examine the effects of varying dose levels of pharmacologic interventions for psychiatric disorders. We formulate our model in context of our PET ethanol study with the general methodology also pertaining to fMRI studies with similar designs, requiring only slight modifications. The remainder of this paper is organized as follows. In the Materials and Methods section, we begin with a description of the primary objective and the design of the ethanol study. We then describe the mixed effects spline models, distinguishing between regression splines and smoothing splines. We also demonstrate how to use the estimates from the fitted spline model to identify brain clusters with similar response profiles. Both the regression spline and smoothing spline models are extended to incorporate interaction effects between the dose-dependent response function and other factors such as experimental condition/task. The Results and Discussion section illustrates the application of our proposed modeling framework using the ethanol study. We also compare the performance of the proposed spline models against polynomial regression models through a simulation study. Finally, we conclude with a discussion.

MATERIALS AND METHODS

1. Description of experimental data

To illustrate our proposed spline models, we use data from an [^{15}O] H_2 PET cerebral blood flow study examining localized changes in brain activity associated with ethanol administration. Ethanol use, abuse, and dependence has existed for thousands of years, yet the mechanisms of action mediating its complex effects on behavior remain poorly understood.

In vivo neuroimaging studies have helped to elucidate aspects of the neurophysiological mechanisms underlying ethanol's impact on brain function (Volkow et al., 1988). In our study, we use [^{15}O]H $_2$ PET during passive ethanol administration to investigate the changes in neural activity that mediate the dose-dependent effects of ethanol. The study includes 16 healthy adult male volunteers, ages 20 to 39 years.

During the study, subjects initially received infusions of a 0.1g/kg glucose solution (placebo) over a 10 minute period. Subsequently, ethanol was administered intravenously at two increasing dose levels, including 20 minutes of small dose (0.2g/kg) and 40 minutes of moderate dose (0.4g/kg). Between any two stages, there was a 10 minute break in infusion. To monitor blood alcohol concentrations (BAC), blood samples were drawn every 5 minutes, beginning immediately after the first dose of ethanol. In this study, BAC was recorded in integer values and ranged from 0mg/ml to 78mg/ml. During the ethanol administration, Regional cerebral blood flow (rCBF) measures of brain activity were determined from 90 second single-frame images acquired following ten serial intravenous injections of 25–35 mCi of [^{15}O]H $_2$. Ten PET images of rCBF were acquired at 10 min intervals over a 100 minute study session that spanned the three stages of dose levels and two 10-minute breaks. Images were reconstructed using a measured attenuation correction (68Ge/68 Ga transmission scans). PET images for each subject were aligned and resliced [Woods et al., 1998a] and spatially normalized to a population-representative PET atlas [Woods et al., 1998b] centered in Talairach stereotaxic coordinates [Talairach and Tournoux, 1988], smoothed with a 9 mm Gaussian filter and normalized for global blood flow.

2. Mixed effects spline models

2.1 Mixed effects regression spline models for dose-dependent neural processing

—We propose the use of mixed effects regression spline models to characterize distributed patterns of localized brain responses to varying dose levels. Corresponding to the ethanol study, we present our model for a PET study and later comment on modifications for fMRI studies. To set notation, let $i = 1, \dots, N$ index subjects, $s = 1, \dots, S$ represent scans, and $v = 1, \dots, V$ index voxels. Define $Y_{is}(v)$ as the rCBF measurement for the i th subject at location (voxel) v at scan s , and x_{is} is the level of the stimulus associated with scan s of subject i .

We model the subject's regional brain activity in terms of the stimulus level using the following mixed effects regression spline model,

$$Y_{is}(v) = \eta_v(x_{is}) + \gamma(v) (g_{is} - \bar{g}_{\dots}) + \mathbf{U}_{is}\beta(v) + d_{i0}(v) + \varepsilon_{is}(v), \quad (1)$$

where the fixed effect $\eta_v(x_{is})$ is a regression spline modeling the effects of the stimulus level x_{is} on brain activity at voxel v , g_{is} is the global blood flow of subject i at scan s and $\bar{g}_{\dots}$ is its mean across all subjects and scans, $\gamma(v)$ is the fixed effects parameter corresponding to the mean-centered global blood flow. We include the global rCBF term in the model to control for the variability in the global rCBF across scans that may confound the estimation of the effects of the stimulus. Design matrix $\mathbf{U}_{is}$ includes covariates of interests other than the stimulus and $\beta(v)$ is the vector of parameters associated with $\mathbf{U}_{is}$. $d_{i0}(v)$ is a subject-specific random effect, and we assume $d_{i0}(v)$ follows a normal distribution $N(0, \sigma_d^2(v))$. $\varepsilon_{is}(v)$ represents random error such that $\varepsilon_{is}(v) \sim N(0, \sigma_e^2(v))$. The random intercept $d_{i0}(v)$ and the error term $\varepsilon_{is}(v)$ are assumed to be independent of each other and are also independent across subjects. Inclusion of the subject random effect takes account of the between-subject variation in rCBF and also accounts for within-subject correlations among the serial rCBF measurements obtained from the same subject.

Regression splines are popular tools for performing nonparametric curve estimation. Essentially, a regression spline is a piecewise polynomial function. We divide the covariate space, here the dose range, into intervals and fit a local polynomial curve within each interval. The continuity of the overall curve across intervals is obtained by using a truncated power basis. We express the regression spline $\eta_v(x_{is})$ in model (1) as

$$\eta_v(x_{is}) = \alpha_0(v) + \alpha_1(v)^* x_{is} + \cdots + \alpha_p(v)^* x_{is}^p + \sum_{k=1}^K \alpha_{p+k}(v) (x_{is} - \kappa_k)_+^p, \quad (2)$$

where $p \geq 1$ is an integer representing the highest order polynomial, the points $\kappa_k, k = 1, \dots, K$, represent knots selected to divide the covariate space, $(x_{is} - \kappa_k)_+ = \max(0, x_{is} - \kappa_k)$ is the truncated power basis, and $\alpha(v) = (\alpha_0(v), \alpha_1(v), \dots, \alpha_{p+K}(v))^T$ is a set of unknown parameters relating the basis functions to the response. From (2), we see that the regression spline is a piecewise polynomial curve. Between two adjacent knots, the spline is a p th degree polynomial and the use of the truncated basis enables the spline to have continuous derivatives up to $p - 1$ order at the knots. A higher order power basis, i.e. larger p , leads to a smoother regression spline. In practice, the most widely used regression splines are piecewise linear with $p = 1$ and piecewise cubic polynomials with $p = 3$. In addition to the truncated power basis, other basis functions for regression splines also exist, such as those for B-splines and radial basis functions (Green and Silverman, 1994). The truncated power basis is very popular due to its simplicity and generally good performance.

An important task in fitting a regression spline is to select the number and placement of knots. Knots are selected from the distinct values of the covariate being modeled. The number of knots depends on the characteristics of the data such as the observed covariate values and the complexity of the underlying response function (Ruppert, 2002). The number of knots can be determined by using cross-validation (Ruppert, 2002) or the Akaike Information Criterion (AIC) (Atilgan, 1988). The computations for these procedures may be time-consuming for imaging data if one considers all possible numbers of knots. In practice, regression splines generally require a small number of knots, with 3–10 knots typically sufficing (Abrahamowicz et al., 1992). Hence, one could fit regression splines at each voxel, with the number of knots ranging from 3–10, and then identify the optimum number of knots that has the minimum AIC for each voxel. Since the observed covariate values (stimulus levels for our data) are the same for all voxels, the optimum number of knots is usually very close across voxels. To increase the comparability of the fitted regression splines across various brain areas, we propose to select a global optimal number of knots by minimizing the overall AIC statistic that is summarized across the brain. In the special cases where the optimum number of knots is quite variable across voxels, one can use the voxel-specific number of knots determined by the AIC statistic, which allows the fitted regression splines to provide a more tailored fit for each voxel, especially for voxels with extreme response functions. However, varying number of knots reduces the comparability of the splines across voxels. After deciding on the number of knots, we need to select the locations of the knots. A general guideline is that the placement of knots should appropriately capture the distribution of the associated covariate. For example, it is suggested that the best placement is to have about equal number of data points between knots (Breiman, 1988) and hence one may place knots at equally spaced quantiles of the observed covariate values (Ngo and Wand, 2004). Since the covariate values are the same across voxels, we specify the same placement of the knots for all brain areas.

The proposed mixed effects regression spline model in (1) not only allows us to flexibly model the dose-dependent effect of the stimulus but also provides a convenient framework for investigating the effects of other factors on brain function while controlling for the effects of the stimulus and subject variability. For example, subjects may be asked to perform certain

tasks during the administration of the stimulus, and researchers may have an interest in the task-related effects on brain function after taking into account the current stimulus level. This can be achieved by incorporating the task condition of each scan into the design matrix U_{is} in model (1), and the corresponding parameters in $\beta(v)$ reflect the marginal effects of the tasks on brain activity controlling for the current stimulus level.

2.2 Secondary analyses based on the mixed effects regression spline model—

The fitted regression spline model reveals a dynamic profile of regional brain activity associated with varying stimulus levels. From the fitted regression spline model, we can readily estimate the brain activity at any stimulus level within the observed range, which is a great advantage over correlation analysis and ANOVA. The proposed mixed effects regression spline model also offers us a powerful tool to derive statistics for measuring and testing specific characteristics in brain response to the stimulus. For example, we can use the parameter estimates from the fitted regression spline model to construct a conventional t-statistic map to test the localized differences in brain activity between two levels of the stimulus; or we can evaluate the rate of change in brain activity in response to the increasing stimulus levels by examining the slope of the fitted spline at each voxel.

Another objective in dose-dependent neuroimaging studies involves identifying groups of brain areas with similar response patterns. To address this question, we can apply results from the mixed effects regression spline model to spatially group brain regions into clusters in such a way that voxels within the same cluster exhibit similar patterns of alterations in response to the stimulus. From the definition of the regression spline model in (2), we can see the shape of the response patterns are fully determined by the vector of parameters $\alpha(v)$ after we specify the locations of the knots. Hence, one can spatially classify voxels based on the vector $\alpha(v)$ using classification methods such as cluster analysis (Bowman et al., 2004). This approach treats all areas of the response profile equally in classifying the brain areas. In cases where certain areas in the response profile are of little interest or have too much noise to be estimated reliably, we can characterize the response pattern by subsampling several key points from the response profile. Specifically, we can either choose stimulus levels that are associated with clinical interpretations or select several representative levels within the observed range of the stimulus. To control for the difference in the baseline activity across voxels, we evaluate the brain activity at selected stimulus levels $a_1, \dots, a_q$ relative to a baseline level a_0 . Based on model (1), the difference in the brain activity between a stimulus level of $a_j (j = 1, \dots, q)$ and a base level a_0 is $\eta_v(a_j) - \eta_v(a_0)$, which can be written as a linear combination of parameters in model (1), i.e. $C_j^T \alpha(v)$ where C_j is a vector of constant coefficients dependent on the contrasting levels a_j and a_0 . Since the raw contrast $C_j^T \alpha(v)$ depends on the level of localized brain activity which varies significantly across the brain, we propose a normalized contrast,

$$t_j(v) = \frac{C_j^T \alpha(v)}{\sqrt{C_j^T \text{var}_v(\alpha) C_j}}.$$

The contrast statistic $t_j(v)$ has the same asymptotic distribution at all voxels under the null hypothesis and hence is comparable across different brain regions. We then characterize a voxel's response profile with a vector of normalized contrasts $T(v) = (t_1(v), \dots, t_q(v))^T$ at selected stimulus levels $a_1, \dots, a_q$. This vector of contrasts depicts the relative activation of voxel v at selected stimulus levels. A cluster analysis could be performed on this vector $T(v)$ to identify groups of voxels that display similar response profiles.

2.3 Mixed effects smoothing spline models for dose-dependent neural processing—

The proposed mixed effects regression spline model is convenient to fit in

practice, but suffers from the limitation that it requires an analyst to select the number and placement of knots which is a time-consuming process for imaging data. As an alternative, we consider the use of a smoothing spline model to characterize the brain activity in response to a stimulus. Smoothing spline is a spline method that avoids knot selection by treating every distinct covariate value as a knot and hence using the maximum set of knots. The complexity or smoothness of a smoothing spline is controlled by a single parameter. In the following, we first give a brief introduction of smoothing spline and then present a mixed effects smoothing spline model for modeling the dose-dependent effect of a stimulus on brain activity. Estimation of the proposed mixed effects smoothing spline model can be performed under the linear mixed model framework through reparameterization.

2.3.1 Smoothing spline: Suppose we want to model observed brain activity measurements $y_j (j = 1, \dots, r)$ in terms of their corresponding stimulus levels $x_j (j = 1, \dots, r)$. Fitting a smoothing spline involves finding a smooth curve $\hat{f}(x)$ to estimate brain activity measurements based on the stimulus level x . There are two major criteria in fitting a smoothing spline: 1) the fitted spline curve should be close to the observed brain activity measurements and 2) the spline curve should be sufficiently smooth. Considering all functions $f(x)$ that are twice-differentiable, the smoothing spline $\hat{f}(x)$ that is optimum in terms of the two criteria is the one that minimizes the following penalized residual sum of squares (RSS) (Green and Silverman, 1994),

$$RSS(f, \lambda) = \sum_{j=1}^r \{y_j - f(x_j)\}^2 + \lambda \int \{f''(x)\}^2 dx. \quad (3)$$

The RSS reflects the two objectives in fitting a smoothing spline. The first term measures the closeness of the curve to the data using a squared error loss function, and the second term penalizes curvature, or roughness, in the curve. The smoothing parameter λ is a non-negative quantity that balances these two objectives and controls the smoothness of curve. When λ equals zero, $\hat{f}(x)$ is a piecewise cubic polynomial that interpolates observed measurements $y_1, y_2, \dots, y_r$; as $\lambda \rightarrow \infty$, $\hat{f}(x)$ reduces to the simple least squares line fit to the data, with $\hat{f}''(x) = 0$ everywhere. The smoothing parameter can be usually chosen using the cross-validation approach (Green and Silverman, 1994). Smoothing splines have a knot at every distinct value of the covariate (the stimulus level x for our data) and hence do not require the selection of the number and placement of knots, which is an advantage over regression splines. Obviously, treating every covariate value as a knot results in over-parameterization. The penalty term $\lambda \int \{f''(x)\}^2 dx$ in the objective function (3) remedies this problem by penalizing the roughness of the curve caused by over-parameterization and inducing restrictions among the parameters in smoothing splines. Previous work (Green and Silverman, 1994) has shown that the unique optimizer $\hat{f}(x)$ that minimizes (3) is a natural cubic spline with knots at the distinct values of the observed stimulus levels $x_j (j = 1, \dots, r)$ (O'Sullivan et al., 1986). A natural cubic spline is a smooth curve that is piecewise cubic polynomial between adjacent knots and continuous up to its second derivative at each knot. Beyond the boundary knots, a natural cubic spline is forced to be linear to avoid wild behavior of cubic polynomials beyond boundaries. Green and Silverman (1994) provide a detailed description of the natural cubic spline.

2.3.2 The mixed effects smoothing spline model: To present our smoothing spline model, we use similar notations as in the regression spline model. Denote $Y_{is}(v)$ as the brain activity measurement for the i th subject at location (voxel) v at scan s , and x_{is} is the level of the stimulus associated with scan s of subject i . To model the dose-dependent effect of the stimulus and to take into account of within-subject correlations among the repeated measurements, we propose the following mixed effects smoothing spline model,

$$Y_{is}(v) = f(x_{is}) + \gamma(v) (g_{is} - \bar{g}_{\dots}) + \mathbf{U}_{is} \beta(v) + d_{i0}(v) + \varepsilon_{is}(v), \quad (4)$$

where $f(x_{is})$ is a twice-differentiable smooth function, $\gamma(v)(g_{is} - \bar{g}_{...})$ adjusts for global blood flow changes, U_{is} includes covariates other than the stimulus level and is associated with the parameter $\beta(v)$. $d_{i0}(v)$ is a subject-specific random effect that follows a normal distribution $N(0, \sigma_d^2(v))$, and $\varepsilon_{is}(v)$ is the random error which follows $N(0, \sigma_e^2(v))$. The random intercept $d_{i0}(v)$ and the error term $\varepsilon_{is}(v)$ are assumed to be independent of each other and are also independent across subjects. Inclusion of the subject random effect terms takes account of the between-subject variation in brain activity measurements and helps to account for within-subject correlations among the repeated measurements obtained from the same subject.

As we recall from the previous section, the smoothing spline is estimated by minimizing the lack of fit of the curve and penalizing the roughness of overfitting. Consequently, the smooth function f and the fixed effects parameters $\gamma(v)$ and $\beta(v)$ in model (4) can be estimated by maximizing the following penalized likelihood

$$-\frac{1}{2} \sum_{i=1}^N \sum_{s=1}^S \{ \ln(\sigma_d^2 + \sigma_e^2) + (\sigma_d^2 + \sigma_e^2)^{-1} [Y_{is}(v) - f(x_{is}) - \gamma(v)(g_{is} - \bar{g}_{...}) - U_{is}\beta(v)]^2 \} - \frac{\lambda}{2} \int \{f''(x)\}^2 dx. \quad (5)$$

The penalized likelihood is composed of two terms which reflect the two objectives in fitting smoothing splines. The first term is the likelihood of the smoothing function and parameters based on the observed data, which boils down to the mean squared error that measures the lack of fit between the observed brain activity measurements and the smoothing spline model. The second term $\int \{f''(x)\}^2 dx$ penalizes the roughness of the curve. The smoothing parameter $\lambda \geq 0$ controls the balance between the goodness-of-fit of the model and the roughness of function $f(x)$. Increasing the smoothing parameter λ results in more smoothed fit. The smooth function estimator $\hat{f}(x)$ that maximizes the penalized likelihood (5) is a natural cubic spline (O'Sullivan et al., 1986).

2.3.3 Estimation for the mixed effects smoothing spline model: Estimation of the parameters in the mixed effects smoothing spline model (4), although seemingly complicated, can be performed under the familiar framework of linear mixed models after appropriate reparameterization. In the following, we re-express model (4) in terms of a modified linear mixed model. Let $\mathbf{x}^0 = (x_1^0, \dots, x_r^0)^T$ with $x_1^0 < \dots < x_r^0$ be a vector of ordered distinct values of the observed stimulus levels x_{is} ($i = 1, \dots, N, s = 1, \dots, S$) and let $\mathbf{N}_i = (\mathbf{N}_{i1}, \dots, \mathbf{N}_{is})^T$ be the $S \times r$ incidence matrix mapping $\mathbf{x}_i = (x_{i1}, \dots, x_{is})^T$ to $\mathbf{x}^0$, i.e. $\mathbf{x}_i = \mathbf{N}_i \mathbf{x}^0$ and the s th row $\mathbf{N}_{is}^T$ is a $1 \times r$ vector such that $x_{is} = \mathbf{N}_{is}^T \mathbf{x}^0$. Define $\mathbf{f} = (f(x_1^0), \dots, f(x_r^0))^T$ as the vector of the smoothing spline evaluated at the r ordered distinct values of the covariate. Based on Green (1987), the smoothing spline function f at each voxel can be written via a one-to-one linear transformation as

$$\mathbf{f} = \mathbf{T}\delta + \mathbf{B}\mathbf{a} \quad (6)$$

where δ and $\mathbf{a}$ are voxel-specific vectors of parameters with dimensions of 2 and $r - 2$ respectively, $\mathbf{T} = [1, \mathbf{x}^0]$ is a $r \times 2$ matrix, and $\mathbf{B}$ is a $r \times (r - 2)$ matrix defined based on the distances between adjacent distinct values in $\mathbf{x}^0$ (Definition of $\mathbf{B}$ is presented by equation (A4) in Appendix A). Through the linear transformation in (6), we can re-write the mixed effects smoothing spline model in (4) as the following modified linear mixed model,

$$Y_{is}(v) = \mathbf{X}_{is}^* \beta^*(v) + \mathbf{Z}_i^* \mathbf{b}_i^*(v) + \varepsilon_{is}(v) \quad (7)$$

where $\mathbf{X}_{is}^* = [g_{is} - \bar{g}_{...}, U_{is}, \mathbf{N}_{is}^T \mathbf{T}]$ is the design matrix for the fixed effects; $\mathbf{Z}_i^* = [\mathbf{N}_{is}^T \mathbf{B}, 1]$ is matrix for the random effects. $\beta^*(v) = [\gamma(v), \beta^T(v), \delta^T(v)]^T$ contains the regression coefficients for the

fixed effects. $\mathbf{b}_i^*(v) = [\mathbf{a}^T(v), d_{i0}(v)]^T$ contains mutually independent random effects, where $\mathbf{a}(v)$ is a vector of random effects of dimension $r - 2$ such that $\mathbf{a}(v) \sim N(0, \tau \mathbf{I})$ with $\tau = 1/\lambda$. In Appendix A, we provide details that establish the equivalence between the mixed effects smoothing spline model (4) and the linear mixed model (7).

Through the above reparameterization, the mixed effects smoothing spline model (4) can then be estimated by fitting the linear mixed model (7) using standard linear mixed model procedures in existing statistical software such as SAS (Pedan, 2003; Zhang et al., 1998). In particular, the inverse of the smoothing parameter $\tau = 1/\lambda$ appears as the variance for a random effect term in the linear mixed model and is conveniently estimated using restricted maximum likelihood method (REML) along with the other variance components. It has been shown that the REML estimator of the smoothing parameter is identical to the generalized maximum likelihood (GML) estimator proposed by Wahba (1985). The REML estimation of the smoothing parameter is more computationally efficient than alternative estimation approaches such as cross-validation (CV) (Rice and Silverman, 1991) and generalized cross-validation (GCV) (Wahba, 1985). Another major advantage of the presented linear mixed model estimation approach is that it provides simultaneous estimation of the smoothing spline, other fixed effects (such as the global normalization term) and subject random effects. Alternative estimation methods for smoothing splines do not provide easy accommodation of the other factors.

2.4 Extensions of the mixed effects spline models: incorporating interaction

effects—The mixed effects spline models in (1) and (4) estimate the main effect of the dose level. Some imaging studies may employ more complex experimental designs which require the study of dose-dependent effects under varying experimental conditions/tasks. For example, consider the ethanol data example in which scans are obtained across increasing BAC levels. If the scans were collected both during a resting state and during a task that engaged cognitive processes, it would be important to characterize the BAC-dependent profiles of measured brain activity separately for each task. To estimate dose-dependent effects under varying experimental conditions/tasks, we extend the proposed mixed effects spline models to incorporate interaction effects between the splines and experimental conditions/tasks. One can determine whether the dose-dependent effect of the stimulus is homogenous across varying experimental conditions/tasks by testing the significance of the interaction effects. We provide the details of the extended mixed effects spline models and the hypothesis tests for the interaction effects in Appendix B.

RESULTS AND DISCUSSION

In this section we present the results from application of the mixed effects spline models to the PET study of ethanol. We also discuss the results from a simulation study that compared the performance of the proposed spline models against polynomial regression for voxels with different response patterns.

3.1 Application to the ethanol study

We illustrate the use of the regression and smoothing spline models using PET rCBF data from the ethanol study. The primary interest in the study is to model the effect of ethanol on brain function and to identify brain regions with similar response patterns to ethanol. Secondly, researchers are also interested in whether the rate of ethanol consumption, measured by the rate of change in BAC, affects the brain. The shape of the brain response pattern to ethanol is unknown and varies considerably across different brain areas. Figure 1 presents the observed data smoothed by loess curves at four voxels, depicting different relationships between rCBF measurements and BAC levels. The varying response patterns cannot be adequately accommodated by standard methods such as polynomial regression. Therefore, we apply the

proposed mixed effects spline models for flexible estimation of the dose-dependent neural processing responses to ethanol.

3.1.1 Results from the mixed effects regression spline model—We first considered the mixed effects regression spline model. We modeled the effect of ethanol on brain function as follows,

$$Y_{is}(v) = \eta_v(BAC_{is}) + \gamma(v)(g_{is} - \bar{g}_{...}) + d_{i0}(v) + \varepsilon_{is}(v), \quad (8)$$

where $\eta_v(BAC_{is})$ is the regression spline defined as follows

$$\eta_v(BAC_{is}) = \alpha_0(v) + \alpha_1(v) * BAC_{is} + \sum_{k=1}^K \alpha_{1+k}(v)(BAC_{is} - \kappa_k)_+$$

For illustration, we used a linear basis functions with $p = 1$ so that the regression spline is a continuous piecewise linear function. One could also use a higher order polynomial basis such as the cubic basis to obtain a smoother spline curve. For the other terms in model (8), g_{is} is the global blood flow of subject i at scan s and $\bar{g}_{...}$ is its mean across all subjects and scans, $\gamma(v)$ is the fixed effects parameter corresponding to the mean-centered global blood flow, $d_{i0}(v)$ is a subject-specific random effect that follows $N(0, \sigma_d^2(v))$ and $\varepsilon_{is}(v)$ is the random error following $N(0, \sigma_e^2(v))$. The subject random intercept $d_{i0}(v)$ and random error $\varepsilon_{is}(v)$ are assumed to be independent of each other and are also independent across subjects. Inclusion of the subject random effects takes account of the between-subject variation in rCBF and helps to account for within-subject correlations among the serial rCBF measurements obtained from the same subject.

To determine the number of knots, we fit regression spline models at each voxel using the number of knots ranging from 3 to 10 and recorded the optimum number producing the minimum AIC statistic. The results across voxels indicated that the optimum number of knots at most voxels laid within the small range of 5–7 and the global optimum number of knots that minimized the overall AIC was 6. To increase the comparability of the fitted regression splines across various brain areas, we chose $K = 6$ for all voxels. The locations of the 6 knots were placed at equally spaced quantiles of the empirical distribution of the observed BAC values so that there were approximately equal number of data points between knots (Ngo and Wand, 2004).

In our study, there was an interest in grouping voxels based on the similarity of their response pattern to ethanol. Using estimates from the regression spline model (8), we performed a cluster analysis on a vector of statistics that characterized the response profile of each voxel. Four representative BAC levels were selected within the range of the observed BAC: 21 mg/ml, 37 mg/ml, 58 mg/ml and 70 mg/ml. We calculated the normalized contrast statistics

$$t_j(v) = \frac{\mathbf{C}_j^T \hat{\alpha}(v)}{\sqrt{\mathbf{C}_j^T \text{var}_v(\hat{\alpha}) \mathbf{C}_j}} \quad (j=1, \dots, 4.)$$

comparing each of the four BAC levels to the baseline BAC 0mg/ml. Here, $\hat{\alpha}(v) = [\hat{\alpha}_0(v), \dots, \hat{\alpha}_{p+K}(v)]^T$ is the vector of the estimated parameters in the regression spline $\eta_v(BAC_{is})$ and $\text{var}_v(\hat{\alpha})$ is its associated estimated variance based on model (8), $\mathbf{C}_j$ is the vector of the contrast coefficients corresponding to the j th comparison. A voxel's response profile to ethanol was characterized by a vector of the normalized contrasts $\mathbf{T}(v) = (t_1(v), \dots, t_4(v))^T$. This vector of contrasts depicted the relative brain activation of voxel v at selected BAC levels compared to the baseline. We then performed a cluster analysis on the vector $\mathbf{T}(v)$. We employed a hybrid clustering approach that starts with the K -means algorithm (MacQueen, 1967) where the initial clusters are individual voxels and then switches to Ward's

hierarchical clustering (Bowman et al., 2004) after the number of clusters is reduced to a specified level. For the hierarchical clustering, we applied the Pseudo-T² statistic as a stopping rule to determine the number of clusters, resulting in 12 clusters. Figure 2 depicts the brain regions and within-cluster mean contrasts for two clusters of particular interest. Cluster A consisted of voxels in the anterior cingulate and medial frontal gyrus. The mean contrasts within Cluster A show these areas exhibited significant increases in brain activity with rising BAC, especially when BAC increased from baseline to 37 mg/ml. Cluster B mainly included voxels in the cerebellum and occipital lobe which demonstrated significant reductions in rCBF with rising BAC especially when BAC increased from the baseline to 58mg/ml; this finding is consistent with results from previous studies (Volkow et al., 1988).

Another research objective in the study is to determine how the rate of change in BAC affects brain function. In our study, ethanol was administered in a continuous manner through the study session and BAC changed over time through the study. Hence ethanol-related brain function alterations at a particular time point were attributable to two sources: the effect from the current level of BAC and the effect associated with the rate of increase in BAC at that time. To accurately investigate how the rate of increase in BAC affects brain function, we need to control for the effect from the current level of BAC. Hence, we considered the following model

$$Y_{is}(v) = \eta_v(BAC_{is}) + d_{i0}(v) + \gamma(v)(g_{is} - \bar{g}_{...}) + \beta(v)\Delta BAC_{is} + \varepsilon_{is}. \quad (9)$$

Model (9) controls for the effect of BAC with the regression spline model $\eta_v(BAC_{is})$, and the additional covariate of interest ΔBAC_{is} represents the rate of change in BAC scan s . Here, we define ΔBAC_{is} as $BAC_{is} - BAC_{is-}$, where BAC_{is-} is the last BAC measured prior to BAC_{is} . The parameter $\beta(v)$ represents is the marginal effect of rate of change in BAC on the brain activity in voxel v , after controlling for the current level of BAC. If one does not include the regression spline $\eta_v(BAC_{is})$ in model (9), the parameter $\beta(v)$ would inaccurately reflect the combined effects of BAC_{is} and ΔBAC_{is} . This would result in an overestimation of the effect of the rate of change in BAC on brain function, especially when BAC_{is} and ΔBAC_{is} exhibit strong positive correlations. Figure 3 identifies the brain areas that we found to be statistically significantly associated with the rate of change in BAC. Specifically, regions that are activated with the rapid increase in BAC include the left temporal gyrus and the right inferior temporal gyrus. Regions that are deactivated with rapid increase in BAC include the inferior frontal gyrus.

3.1.2 Results from the mixed effects smoothing spline model—To avoid the need for knots specifications, we also applied the following mixed effects smoothing spline model to describe the dose-dependent effects of ethanol,

$$Y_{is}(v) = f(BAC_{is}) + \gamma(v)(g_{is} - \bar{g}_{...}) + d_{i0}(v) + \varepsilon_{is},$$

where $f(BAC_{is})$ is the smoothing spline with BAC. We estimated parameters in the mixed effects smoothing spline model under the linear mixed model framework through the reparameterization presented in (7).

For illustration, we demonstrate estimated smoothing splines at selected brain areas that showed significant alterations either when a subject initiated ethanol intake or when BAC started to approach the legal limit. In particular, we selected four voxels from brain regions exhibiting significant alterations when BAC increased from 0 mg/ml to 20 mg/ml or when BAC increased from 58 mg/ml to 70 mg/ml based on conventional t-test. The fitted smoothing splines for the four voxels appear in Figure 4. The smoothing splines not only confirm the results from the conventional t-test in terms of the significant changes between contrasting BAC levels, but also reveal the dynamic activation patterns at these brain areas across the entire range of the observed BAC levels. For example, the voxel depicted in Figure 4(a) shows an

initial deactivation when BAC increases from 0 to 40 mg/ml, followed by a gradual rise in brain activity beyond that point. The voxel in Figure 4(b) shows an initial activation, but levels off after BAC exceeds 30 mg/ml. The voxels in Figures 4(c) and 4(d) do not show significant alterations until BAC exceeds 60 mg/ml, after which level the voxels exhibit precipitous changes in brain activity. Compared to the conventional t-test which can only reveal the difference in brain activity between two specific BAC values, the spline model depicts the complete profile of the localized brain activity at various BAC levels, offering a much richer characterization in studying the dose-dependent effects of the stimulus on the brain. In Figure 4, we also plot the estimated regression splines. In comparison to smoothing splines, the fitted regression splines demonstrate similar response patterns for each of the voxels but appear to be a bit rougher.

3.2 A simulation study

We conducted a simulation study to evaluate the performance of our proposed mixed effects spline models under various response patterns. The accuracy of the proposed spline models were compared with that of polynomial regression models. We generated 200 data sets each composed of 10 subjects with 10 repeated measurements of brain activity in response to a stimulus. The data for subject i ($i = 1, \dots, 10$) at scan s ($s = 1, \dots, 10$) was generated from the following model

$$Y_{is}(v) = f_v(X_{is}) + \gamma(g_{is} - \bar{g}_{\dots}) + d_{i0}(v) + \varepsilon_{is},$$

where $f_v(\cdot)$ is the response function at voxel v and is defined later. We simulated the stimulus variable X from a uniform distribution ranging between 0 and 18, representing dose levels. Based on the estimates of the ethanol study, we generated the centered global blood flow ($g_{is} - \bar{g}_{\dots}$), the subject random intercepts $d_{i0}(v)$ and the random errors ε_{is} from zero-mean Gaussian distributions with standard deviation of 5. To evaluate the ability of the proposed models to capture varying response patterns, we simulated data for four voxels with different response patterns. The response curve $f(X)$ for the four voxels are:

- A. $f_1(X) = 36 + 1.6X - 0.132X^2 + 0.006X^3$,
- B. $f_2(X) = 45 + 2\sin(\pi*(X-5)/10) + 5.2*\sin(2\pi*(X-5)/25 + 5.8) - 3.8*\sin(2\pi*(X-5)/10 + 5.5)$,
- C. $f_3(X) = 20 + 1.2X + 8*\sin(\pi*(X-5)/10) + 8*\sin(2\pi*(X-5)/25 + 8.8)$,
- D. $f_4(X) = \begin{cases} 40 & X < 5 \\ 4X + 20 & 5 < X < 10 \\ -0.4X^2 + 8X + 63.75 & 10 < X < 15 \\ 50 & 15 < X < 18 \end{cases}$.

The response function of voxel (A) is a quadratic curve and represents a simple response shape. The response functions of voxels (B) – (D) are non-linear curves representing more complex, yet realistic, dose-dependent response patterns. For example, voxel (B) features an initial increase followed by a brief leveling-off period and then a further increase to the maximum; voxel (D) starts with a steady state when the stimulus is below a threshold and exhibits a linear increase thereafter; after reaching the maximum response, the brain activity decreases and finally levels off.

We analyzed the simulated data using the proposed mixed effects regression spline and smoothing spline models. For the regression spline model, we used truncated linear basis. The number of knots was selected based on the AIC statistic and the knots were placed at equally spaced quantiles of the empirical distribution of the stimulus. The smoothing spline models were estimated under the linear mixed model framework where the smoothing parameter was

estimated as the variance component through REML. In comparison to the proposed spline models, we also fit a mixed effects polynomial regression model where the dose-dependent effect was modeled through a cubic function. Figure 5 presents the true curves and the mean estimated curves based on the three models. To quantify the difference between the estimated curves and the true curve, one may be inclined to simply calculating the MSE between the estimates and true values and then averaging across the entire curve to obtain a summary accuracy measure. However, this crude measure is hard to interpret since it depends on the magnitude of the response function. Furthermore when averaging the MSE across the response curve, one doesn't take into account of the varying level of response function across the range of the stimulus. To address these issues, we define a localized relative error statistic which is the ratio of the squared root of the MSE to the true value of the response function at each point of the simulated curve. This localized relative error statistic measures the estimation error as a percentage of the true value. Hence it does not depend on the magnitude of the response function and is comparable across the response curve. We then summarize the overall estimation error by averaging the localized relative error statistic across the range of the simulated curve.

Based on Figure 5, for voxel (A) with a cubic polynomial response pattern, all three models successfully estimated the shape of the response function. For voxels (B)–(D) where the response profiles possessed more complex shapes, the spline models still successfully estimated the actual shapes while the cubic regression model, which was restricted by the parametric assumption on the shape of the curve, was not flexible enough to capture certain important aspects of the response curves. For example, in voxel (B), the polynomial regression model failed to reveal the leveling-off period between the initial increase and the second increasing stage; in voxel (D), the polynomial model could not estimate the two steady states at the two ends of the curve and also failed to accurately estimate the stimulus level where the response curve reached the maximum. Between the regression spline and smoothing spline models, we found the regression spline was slightly less accurate in areas with large curvature such as the peak in voxel (D). The overall relative error for the cubic regression model, regression spline model and smoothing spline model is 0.15%, 0.31% and 0.30%, respectively, for voxel (A); 4.66%, 0.97% and 0.93% for voxel (B); 4.31%, 1.09% and 1.04% for voxel (C); and 4.55%, 1.20% and 1.03% for voxel (D). The simulation results show the proposed spline models are more flexible than the polynomial regression model and are more successful in capturing complex response patterns across different brain areas.

CONCLUSIONS

We propose a class of mixed effects spline models for studying the dose-dependent effect of a stimulus on brain function. Our method models the stimulus effect with a spline curve which offers a flexible framework for capturing various dynamic response patterns across the brain. The collection of localized fitted spline curves allows estimation of the effect of the stimulus at various dose ranges and permits convenient secondary analyses such as clustering brain areas based on the response patterns. Our model also offers a flexible framework to control for relevant factors and accommodates interactions between the spline and other factors, hence providing more accurate estimation of the dose-dependent effects of the stimulus. Furthermore, the proposed modeling framework accounts for between-subject variability in the brain activity measurements by incorporating the subject-specific random effects.

We discuss two types of spline models, including regression splines as well as smoothing splines. Compared to conventional polynomial regression models that impose a global polynomial form on the response profile, regression splines can better characterize dynamic brain response profiles by fitting piecewise polynomials, yielding response curves with shapes that are less restricted. An advantage of regression splines is the straightforward computations,

once the knots are determined. This facilitates straightforward secondary analysis based on the estimated regression splines. The main drawback, however, lies in the difficulty of choosing the number and the position of the knots. Since the observed covariate values are the same across voxels, we choose a common number of knots for all voxels to improve the comparability of the fitted regression splines across various brain areas. Alternatively, one may choose a voxel-specific number of knots which allows the fitted regression splines to provide a more tailored fit. In this paper, we select the number of knots based on the AIC statistic. One need to be aware that AIC may tend to favor more complex models, i.e. larger number of knots, due to its relatively lenient penalty on the number of parameters (Dominici F, et al., 2003). As an alternative, one could consider other information criteria such as the Bayesian information criterion (BIC) (Schwarz, 1978). The second type of spline model that we propose is a mixed effects smoothing spline model that provides smooth dynamic response profiles, without requiring the analyst to specify the number and the placement of knots. The connection between the smoothing spline and the linear mixed model allows estimation of the parameters using a conventional linear mixed model framework. Estimation of the proposed smoothing spline model can therefore be conveniently performed using existing statistical software. For functional neuroimaging data, we recommend the use of the mixed effects smoothing spline model when the main objective lies in characterizing response patterns across the brain because of its greater flexibility and the freedom from the need of knot selection. If one is more interested in performing secondary analysis based on the estimated response curves (such as clustering brain regions according to response patterns), the mixed effect regression spline model would allow more straightforward estimation of statistics for the secondary analysis. Finally, we would like to point out another advantage of regression splines. Compared to smoothing splines, regression splines require more human interventions such as choosing the number and placement of knots, which are often taken as the downside to regression splines because they increase the amount of work and also induce subjectivity into estimation. However, human interventions in regression splines could be beneficial in some cases in the sense that they offer investigators more control over the model. For example, for small data sets, estimation of smoothing splines is generally difficult whereas reasonable estimates could be obtained for regression splines by appropriate specifications of model parameters such as the number and placement of knots (Wahba, 1988).

Applications of spline models are rather limited in the functional neuroimaging literature. Splines have been used in neuroimaging analyses to smooth fMRI time series for removing low frequency noise (Macey et al., 2004; Tanabe et al., 2002) or for reducing temporal autocorrelations (Carew et al. 2003). However, we have not encountered any work that uses splines for flexible modeling of the measured brain activity corresponding to a continuous covariate, here dose level, especially when the covariates are repeatedly measured within each subject. Our proposed modeling framework helps to fill this gap. In particular, smoothing spline models have not been widely applied to neuroimaging data partly due to the computational challenges. In this paper, we present a linear mixed model framework to make inferences for smoothing splines, which will facilitate its applications in imaging studies. Carew et al. (2003) described an inference procedure for smoothing splines, where the smoothing spline is fitted by assuming a fixed smoothing parameter. This procedure is performed repeatedly with a series of smoothing parameters that are chosen somewhat arbitrarily, and the optimal smoothing parameter is then determined by comparing the Generalized Cross-Validation (GCV) scores. As an alternative, we present an inference procedure under the linear mixed model framework that offers several key advantages over the approach by Carew et al. (2003). First, our inference procedure is computationally less expensive because it does not require repeatedly fitting the spline model for different choices of smoothing parameters at each voxel. The smoothing parameter is estimated through the REML in the linear mixed model, and it has been shown that the REML estimator of the smoothing parameter is identical to the generalized maximum likelihood (GML) estimator proposed by Wahba (1985). Kohn et

al. (1991) showed through an extensive simulation study that the REML estimator performs well and often better compared to the GCV estimator. Furthermore, unlike the estimation method in Carew et al. (2003), which can only make inference for the smoothing spline, our inference procedure provides simultaneous estimation for both the smoothing spline and other factors such as global normalization terms, additional covariates and subject-specific random effects. It is typically important to include these factors in the model for accurate estimation of the stimulus effect. In particular, the subject-specific random effect is a crucial factor in imaging studies due to the considerable between-subjects variability in brain activity. Our method provides a unified framework for modeling the smoothing spline, other fixed effects and subject random effects using a linear mixed model, which greatly facilitates estimation.

Corresponding to the motivating ethanol study, we formulate the mixed effects spline models in the context of our PET study. However, our models are also applicable to fMRI studies, with some slight modifications. In fMRI studies, the acquisition of brain scans occurs more frequently than the measurement of the concentration or dose levels of the stimulus, which means we have multiple fMRI scans under a single dosage or concentration level. Furthermore, the observed BOLD response in fMRI reflects the combined effects of the stimulus and the hemodynamic response function. Consequently, the BOLD responses may not be the desirable outcome to model the dose-dependent effect of the stimulus. For fMRI data, we recommend the following modeling scheme:

1. separate the study period into intervals by the time points when the dosage or the stimulus concentration level is measured, and we denote these intervals by $t = 1, \dots, T$.
2. assume a constant level of the stimulus within each interval $\bar{x}(t)$. The interval dosage $\bar{x}(t)$ can be set as an interpolation of the stimulus levels measured at the two ends of the intervals.
3. compute a summary statistic $B_{it}(v)$ based on the BOLD measurements within each of the interval to represent the brain activity during this interval. For example, one can fit a usual GLM model on BOLD measurements where the effects consist of interval-specific intercept terms, i.e. specifying a different intercept for each of the intervals. The estimated intercept for interval t can then be used as the summary statistic $B_{it}(v)$.
4. finally, apply the proposed mixed effects spline models to analyze the summarized brain activity $B_{it}(v)$ in terms of the interval dosage $\bar{x}(t)$.

When introducing the mixed effects spline models in this paper, we focus on modeling the dose-dependent neural responses. However, the proposed models can be applied in more general settings for modeling the effects of any continuous covariates on brain function. The spline models are particularly useful in exploring and revealing varying response patterns across the brain. The estimated response profiles describe the neural processing responses across the entire range of the continuous covariate and provide much more information than conventional test statistics such as the t-statistics, which only represent the contrast between two covariate levels.

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Appendix A. Rewrite the mixed effects smoothing spline model as a linear mixed model

Denote $\mathbf{Y} = (y, \dots, y)^T$ as the repeatedly measured response variable of subject i . $\mathbf{x}_i = (x_{i1}, \dots, x_{iS})^T$ be the vector of the covariate that is modeled nonparametrically using the smoothing spline. Let $\mathbf{x}^0 = (x_1^0, \dots, x_r^0)^T$ be a vector of ordered distinct values of x_{is} ($i = 1, \dots, N$, $s = 1, \dots, S$) and let $\mathbf{N}_i$ be the incidence matrix mapping $\mathbf{x}_i = (x_{i1}, \dots, x_{iS})^T$ to $\mathbf{x}^0$, i.e. $\mathbf{x}_i = \mathbf{N}_i \mathbf{x}^0$. $\mathbf{X}_i$ is a $S \times p$ design matrix that include the fixed effects covariates, $\mathbf{Z}_i$ is a $S \times q$ design matrix that include the random effects covariates. To help demonstrate the connection between the proposed mixed effects smoothing spline model and linear mixed model, we first present the mixed effects smoothing spline model in a more general form as follows

$$\mathbf{Y}_i = \mathbf{X}_i \boldsymbol{\beta} + \mathbf{N}_i \mathbf{f} + \mathbf{Z}_i \mathbf{d}_i + \boldsymbol{\varepsilon}_i, \quad (\text{A1})$$

Where $\boldsymbol{\beta}$ is vector of parameters for the fixed effects, $\mathbf{f} = (f(x_1^0), \dots, f(x_r^0))^T$ is the vector of the smoothing spline evaluated at the r ordered distinct values of the covariate, $\mathbf{d}_i$ include the parameters for the subject-specific random effects with $\mathbf{d}_i \sim N(0, \mathbf{D})$, $\boldsymbol{\varepsilon}_i$ is the vector of random errors with $\boldsymbol{\varepsilon}_i \sim N(0, \sigma^2 \mathbf{I})$. If we rewrite the mixed effects smoothing spline model in (4) in terms of this general form, $\mathbf{X}_i$ consists of the global normalization term and covariates in $\mathbf{U}_{is}$, $\mathbf{Z}_i = \mathbf{1}$ which is an $S \times 1$ vector, $\mathbf{d}_i$ is the subject-specific random intercept d_{i0} . We estimate the fixed effects $\boldsymbol{\beta}$ and $\mathbf{f}$ in (A1) by maximizing the following penalized likelihood,

$$\sum_{i=1}^N \left\{ -\frac{1}{2} \log |\mathbf{V}_i| - \frac{1}{2} (\mathbf{Y}_i - \mathbf{X}_i \boldsymbol{\beta} - \mathbf{N}_i \mathbf{f})^T \mathbf{V}_i^{-1} (\mathbf{Y}_i - \mathbf{X}_i \boldsymbol{\beta} - \mathbf{N}_i \mathbf{f}) \right\} - \frac{\lambda}{2} \int [f''(x)]^2 dx \quad (\text{A2})$$

where λ is the smoothing parameter.

O'Sullivan et al. (1986) showed $\hat{\mathbf{f}}$ that maximizes (A2) is a natural cubic spline. Based on Green (1987), the smoothing spline function $\mathbf{f}$ can be written via a one-to-one linear transformation as

$$\mathbf{f} = \mathbf{T}\boldsymbol{\delta} + \mathbf{B}\mathbf{a} \quad (\text{A3})$$

where $\boldsymbol{\delta}$ and $\mathbf{a}$ are vector of parameters with dimensions of 2 and $r-2$, $\mathbf{T} = [1, x^0]$ is a $r \times 2$ matrix. To define $\mathbf{B}$, we need to first introduce two band matrices $\mathbf{Q}$ and $\mathbf{R}$ defined on the distances between adjacent distinct values of the covariate, i.e. $h_j = x_{j+1}^0 - x_j^0$ for $j = 1, \dots, r$. Following Green and Silverman (1994), $\mathbf{Q}$ is the $r \times (r-2)$ matrix with entries q_{ij} for $i = 1, \dots, r$ and $j = 2, \dots, r-1$ given by

$$q_{j-1,j} = h_{j-1}^{-1}, q_{j,j} = -h_{j-1}^{-1} - h_j^{-1}, q_{j+1,j} = h_j^{-1}, \text{ and } q_{i,j} = 0 \text{ for } |i-j| \geq 2.$$

$\mathbf{R}$ is the $(r-2) \times (r-2)$ symmetric matrix with elements R_{ij} for $i, j = 2, \dots, r-1$ given by

$$R_{ii} = \frac{1}{3}(h_{i-1} + h_i) \text{ for } i = 2, \dots, r-1; R_{i,i+1} = R_{i+1,i} = \frac{1}{6}h_i \text{ for } i = 2, \dots, r-2; \text{ and } R_{ij} = 0 \text{ for } |i-j| \geq 2.$$

Denote the Cholesky decomposition of $\mathbf{R}^{-1}$ as $\mathbf{R}^{-1} = \mathbf{G}\mathbf{G}^T$ and let $\mathbf{L}$ be a $r \times (r-2)$ full-rank matrix such that $\mathbf{L} = \mathbf{Q}\mathbf{G}$. The matrix $\mathbf{B}$ in (A3) is then defined as,

$$\mathbf{B} = \mathbf{L}(\mathbf{L}^T \mathbf{L})^{-1}. \quad (\text{A4})$$

Next, we will show the equivalence of the likelihood function in (A2) to a likelihood of a linear mixed model when we write the smoothing spline $\mathbf{f}$ in terms of its one-to-one linear transformation in (A3). Define a matrix $\mathbf{K} = \mathbf{Q}\mathbf{R}^{-1}\mathbf{Q}^T$. It has been shown the roughness penalty term $\int [f''(x)]^2 dx$ in (A2) is equal to $\mathbf{f}^T \mathbf{K} \mathbf{f}$ (Green and Silverman, 1994). It can be shown from (A3) that $\mathbf{f}^T \mathbf{K} \mathbf{f} = \mathbf{a}^T \mathbf{a}$ by noting that $\mathbf{K} = \mathbf{L}\mathbf{L}^T$ and $\mathbf{L}^T \mathbf{T} = 0$. By inputting (A3) into the penalized likelihood in (A2) and by replacing the roughness penalty term with $\mathbf{a}^T \mathbf{a}$, the penalized likelihood can be rewritten as

$$\sum_i^n \left\{ -\frac{1}{2} \log |\mathbf{V}_i| - \frac{1}{2} (\mathbf{Y}_i - \mathbf{X}_i \boldsymbol{\beta} - \mathbf{N}_i \mathbf{T} \boldsymbol{\delta} - \mathbf{N}_i \mathbf{B} \mathbf{a})^T \mathbf{V}_i^{-1} (\mathbf{Y}_i - \mathbf{X}_i \boldsymbol{\beta} - \mathbf{N}_i \mathbf{T} \boldsymbol{\delta} - \mathbf{N}_i \mathbf{B} \mathbf{a}) \right\} - \frac{1}{2\tau} \mathbf{a}^T \mathbf{a}, \quad (\text{A5})$$

where $\tau = 1/\lambda$. One can immediately see that (A5) is the likelihood for the following linear mixed model

$$\mathbf{Y}_i = \mathbf{X}_i^* \boldsymbol{\beta}^* + \mathbf{Z}_i^* \mathbf{b}_i^* + \varepsilon_i \quad (\text{A6})$$

where $\mathbf{X}_i^* = (\mathbf{X}_i, \mathbf{N}_i \mathbf{T})$ and $\mathbf{Z}_i^* = (\mathbf{N}_i \mathbf{B}, \mathbf{Z}_i)$ are the design matrices for the fixed and random effects, respectively; and $\boldsymbol{\beta}^* = (\boldsymbol{\beta}^T, \boldsymbol{\delta}^T)^T$ are the regression coefficients and $\mathbf{b}_i^* = (\mathbf{a}^T, \mathbf{d}_i^T)^T$ are mutually independent random effects with $\mathbf{a} \sim N(0, \tau \mathbf{I})$ and $\mathbf{d}_i \sim N(0, \mathbf{D})$. Note that the inverse of the smoothing parameter $\tau = 1/\lambda$ is the variance for the random effects $\mathbf{a}$.

Therefore, the mixed effects smoothing spline model (A1) can be written in terms of the linear mixed model (A6). The smoothing parameter and the variance components are estimated with restricted maximum likelihood method (REML).

Appendix B. Extensions of the mixed effects spline models: incorporating interaction effects

Extension of the mixed effects regression spline model for interaction effects

We present an extension for model (1) that incorporates interactions between the dose-dependent effect and a covariate such as experimental conditions/tasks. Suppose we have a covariate w_{is} with L categories, i.e. $w_{is} \in \{1, \dots, L\}$, for scan s of subject i , and that the dose-dependent effect of the stimulus x_{is} may depend on the value of w_{is} . To model the interactions between the stimulus and the covariate w_{is} , we propose to use the following regression spline,

$$\begin{aligned} \eta_v(x_{is}) = & \alpha_0(v) + \sum_{j=1}^p \alpha_j(v) * x_{is}^j + \sum_{k=1}^K \alpha_{p+k}(v)(x_{is} - \kappa_k)_+^p + \sum_{\ell=1}^L w_{is\ell} \left(\alpha_0^\ell(v) + \sum_{j=1}^p \alpha_j^\ell(v) * x_{is}^j \right) \\ & + \sum_{\ell=1}^L w_{is\ell} \left(\sum_{k=1}^K \alpha_{p+k}^\ell(v)(x_{is} - \kappa_k)_+^p \right) \end{aligned} \quad (A7)$$

where $w_{is\ell}$ ($\ell=1, \dots, L$) are a set of indicators for various categories of w_{is} such that $w_{is\ell}$ equals 1 if $w_{is} = \ell$ and 0 otherwise. Since the above regression spline model is over-parameterized, we adopt the constraints that $\alpha_0^1(v) = \dots = \alpha_{p+K}^1(v) = 0$ to make the parameters identifiable (Ruppert, et al., 2003). Based on (A7) and the adopted constraints, the regression spline for the dose-dependent effect of x_{is} when $w_{is} = 1$ reduces to the model in (2) and the regression spline of x_{is} when $w_{is} = \ell$ ($\ell = 2, \dots, L$) is

$$\eta_v(x_{is}) = \alpha_0(v) + \alpha_0^\ell(v) + \sum_{j=1}^p [\alpha_j(v) + \alpha_j^\ell(v)] * x_{is}^j + \sum_{k=1}^K [\alpha_{p+k}(v) + \alpha_{p+k}^\ell(v)](x_{is} - \kappa_k)_+^p.$$

By plugging in (A7) into model (1), we obtain an extended mixed effects regression spline model that can estimate the dose-dependent effect of a stimulus under different values of a covariate, such as experimental conditions/tasks.

When we model the dose-dependent effect of the stimulus using interaction model (A7), one may be interested in testing the significance of the interaction between the dose-dependent response function and the other covariate w_{is} . After accounting for the constraints $\alpha_0^1(v) = \dots = \alpha_{p+K}^1(v) = 0$ for identifiability, the null hypothesis to test the significance of the interaction effects in (A7) is

$$H_0: \alpha_0^\ell(v) = \dots = \alpha_{p+K}^\ell(v) = 0 \text{ for } \ell = 2, \dots, L$$

One could test this hypothesis using the likelihood ratio test (Cox and Hinkley, 1974) which is based on the difference in the log-likelihoods of the main effects model (1) and the interaction model (A7) (Ruppert et al., 2003). Under the null hypothesis, the likelihood ratio statistic follows a Chi-square distribution with a degree of freedom of $(L-1) * (p+K+1)$. In the absence of significant interaction, the effects of the stimulus don't change significantly across different covariate values and one may choose to fit the main effects model (1) to characterize the dose-dependent effect of the stimulus under all conditions.

Extension of the mixed effects smoothing spline model for interaction effects

We now extend the mixed effects smoothing spline model (4) to accommodate interactions between the dose-dependent effect of the stimulus x_{is} and another covariate w_{is} where $w_{is} \in \{1, \dots, L\}$. As an extension to (4), we consider the following model,

$$Y_{is}(v) = f(x_{is}) + \sum_{\ell=2}^L w_{is\ell} \phi_{\ell}(x_{is}) + \gamma(v) (g_{is} - \bar{g}_{\dots}) + \mathbf{U}_{is} \beta(v) + d_{i0}(v) + \varepsilon_{is}(v) \quad (\text{A8})$$

where $w_{is\ell}$ ($\ell = 2, \dots, L$) are a set of indicators for various categories of w_{is} such that $w_{is\ell}$ equals 1 if $w_{is} = \ell$ and 0 otherwise. The functions $f(x)$ and $\phi_{\ell}(x)$ ($\ell = 2, \dots, L$) are smoothing splines where $f(x)$ models the dose-dependent effect of the stimulus when $w_{is} = 1$ and $\phi_{\ell}(x)$ models the deviation between $w_{is} = \ell$ ($\ell = 2, \dots, L$) and $w_{is} = 1$. The functions $\phi_{\ell}(x)$ are centered for the sake of identifiability (Zhang et al., 1998). Here, we set $w_{is} = 1$ as the reference level but one can also choose a different value of w_{is} as the reference level under the same framework. To estimate the extended mixed effects smoothing spline model, we first use similar linear transformation as in (6) to write the smoothing splines f and ϕ_{ℓ} as $f = \mathbf{T}\delta_1 + \mathbf{B}\mathbf{a}_1$ and $\phi_{\ell} = \mathbf{x}_{*}^0 \delta_{\ell} + \mathbf{B}\mathbf{a}_{\ell}$ where δ_1 is a vector of parameters with dimension 2, δ_{ℓ} is a scalar parameter, $\mathbf{x}_{*}^0$ denotes $\mathbf{x}^0$ centered about its mean and $\mathbf{a}_{\ell} = (\ell = 1, \dots, L)$ are independent random effects parameters that follow normal distributions, i.e. $\mathbf{a}_{\ell} \sim N(0, \tau_{\ell} \mathbf{I})$. By plugging in f and ϕ_{ℓ} into (A8), we can re-write the extended mixed effects smoothing spline model as a linear mixed model similar to (7). One can use existing statistical software such as SAS to estimate and make inference for the mixed effects smoothing spline model with interaction terms.

To decide whether the smoothing splines differ significantly between various covariate values, i.e. whether there are any significant interaction effects, we test the null hypothesis that the smoothing splines $\phi_{\ell} = 0$ for $\ell = 2, \dots, L$. Based on the linear transformation of the smoothing spline $\phi_{\ell} = \mathbf{x}_{*}^0 \delta_{\ell} + \mathbf{B}\mathbf{a}_{\ell}$, it is equivalent to testing whether parameters δ_{ℓ} and $\mathbf{a}_{\ell}$ all equal 0. $\mathbf{a}_{\ell}$ follows a zero-mean normal distribution $N(0, \tau_{\ell} \mathbf{I})$, $\mathbf{a}_{\ell} = 0$ means the normal distribution degenerates at the single point 0 which implies the variance component $\tau_{\ell} = 0$. Hence, the null hypothesis for the test of significance for the interaction effects in the smoothing spline model (A8) is

$$H_0 : \delta_{\ell} = 0 \text{ and } \tau_{\ell} = 0 \text{ for } \ell = 2, \dots, L$$

To test this hypothesis, one could use the likelihood ratio test that is based on the difference in the log-likelihoods for the main effects model (4) and the interaction effects model (A8). To decide the null distribution for the likelihood ratio test statistics, we note that the null hypothesis of $\tau_{\ell} = 0$ lies on the boundary of the parameter space because the variance components $\tau_{\ell} \geq 0$. In this case, the null distribution for the likelihood ratio statistic equals a weighted sum of Chi-

square distributions (Shapiro, 1988), which in our case is $\sum_{m=1}^L 2^{-(L-1)} \binom{L-1}{m-1} \chi_m^2$. We would reject the null hypothesis if the likelihood ratio statistic exceeds a specified upper percentile of this mixture Chi-square distribution. When rejecting the null hypothesis, we would conclude that there are significant interaction effects between the dose-dependent effect of the stimulus and the other covariate (such as experimental conditions/tasks) and the extended mixed effects smoothing spline model (A8) would allow us to capture the different response functions under varying values of the other covariate. Otherwise, in the absence of significant interactions, one may choose to fit the main effects model (4) to characterize the dose-dependent effect of the stimulus under all conditions.

Figure 1.

Descriptive plots showing varying dose-dependent response patterns at different voxels in the ethanol study. We depicted the regional cerebral blood flow (rCBF) at four voxels at various blood alcohol concentration (BAC) levels. The observed data were smoothed using the loess curve. The varying response patterns cannot be accommodated by standard methods such as the polynomial regression models and prompt the need to develop methods that more effectively capture dose-dependent neural processing responses.

Cluster A

Cluster B

Figure 2.

Maps of two brain region clusters identified by a cluster analysis for grouping brain locations based on their response patterns to ethanol. Each region is shown with coronal, sagittal and axial slices. The figures on the right column plot the mean contrasts within each of the clusters. Cluster A exhibited increase in brain activity with rising BAC and includes anterior cingulate and medial frontal gyrus. Cluster B demonstrated reduction in brain activity with increasing BAC and mainly includes cerebrum and occipital lobe.

Figure 3. Maps of brain regions that have significant association with the rate of increase in BAC, based on a mixed effects regression spline model. Axial slices are relative to the anterior commissural plane. (a) highlighted regions indicate voxels that have significant positive association with the rate of increase in BAC at $\alpha = 0.005$; (b) highlighted regions indicate voxels that have significant negative association with the rate of increase in BAC at $\alpha = 0.005$.

Figure 4.

Plot of fitted smoothing spline for four selected voxels: (a) with significant decrease when BAC increase from 0 mg/ml to 20 mg/ml (b) with significant increase when BAC increases from 0 mg/ml to 20 mg/ml (c) with significant decrease when BAC increase from 58 mg/ml to 70 mg/ml (d) with significant increase when BAC increases from 58 mg/ml to 70 mg/ml.

Figure 5.

Results from the simulation study comparing the performances of the proposed mixed effects spline models and a cubic regression model in four voxels with varying response patterns. For voxels (A)–(B), we plot the true response curve and the mean estimated curves by the mixed effects regression spline and smoothing spline models and the cubic regression model. All three models provide accurate estimates for voxel (A) with a cubic polynomial response. For more complex response patterns in (B)–(D), the proposed spline models perform better than the cubic regression model.