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Scaled Subprofile Modeling of Resting State Imaging Data in Parkinson's Disease: Methodological Issues

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Abstract

Consistent functional brain abnormalities in Parkinson's disease (PD) are difficult to pinpoint because differences from the normal state are often subtle. In this regard, the application of multivariate methods of analysis has been successful but not devoid of misinterpretation and controversy. The Scaled Subprofile Model (SSM), a principal components analysis (PCA)-based spatial covariance method, has yielded critical information regarding the characteristic abnormalities of functional brain organization that underlie PD and other neurodegenerative disorders. However, the relevance of disease-related spatial covariance patterns (metabolic brain networks) and the most effective methods for their derivation has been a subject of debate. We address these issues here and discuss the inherent advantages of proper application as well as the effects of the misapplication of this methodology. We show that ratio pre-normalization using the mean global metabolic rate (GMR) or regional values from a "reference" brain region (e.g. cerebellum) that may be required in univariate analytical approaches is obviated in SSM. We discuss deviations of the methodology that may yield erroneous or confounding factors.

Keywords

Positron Emission Tomography; Brain imaging; Parkinson's disease; Principal Component Analysis; Multivariate spatial covariance analysis; Global normalization

1. Introduction

Parkinson's disease (PD) usually begins in late middle age and progresses slowly. Diagnosis is difficult because of the small magnitude of changes in regional brain function especially in early clinical stages of the disease. In addition, a variety of distinct, atypical parkinsonian syndromes have also been recognized. These progressive neurodegenerative disorders involve similar brain regions as classical PD – although with different histopathological changes – and can manifest initially with many of the same clinical signs and symptoms (Tolosa et al., 2006). Thus, consistent and diagnostically-specific changes in regional brain function have generally not been evident in resting state images obtained using modalities such as PET, SPECT or fMRI, particularly early in the disease course. Indeed, univariate and multivariate analytical tools have had varied success in delineating regional and

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systems-level abnormalities relating to the inherent pathology of these and other neurodegenerative conditions (e.g., Eckert et al., 2005; Mosconi et al., 2008; Habeck et al., 2008; Tang et al., 2010b). In contrast to multivariate methods of analysis, univariate methods cannot partition the sources of variance into disease and non-disease related components, where the latter can often overshadow the former, and do not examine the complex interconnectivity of voxels that is inherent in brain function.

Resting state metabolic imaging studies in PD provide a useful example of the potential utility of multivariate analysis in the clinical investigation of neurodegenerative disorders and their response to therapeutic interventions (see e.g., Eidelberg, 2009; Poston and Eidelberg, 2009). Using the scaled subprofile model (SSM, Moeller and Strother, 1991; Alexander and Moeller, 1994), a form of multivariate analysis based on principal components analysis (PCA), we identified a characteristic spatial covariance pattern associated with PD. This Parkinson's disease-related pattern (PDRP, Fig. 1) was first described in regional metabolic PET data (Eidelberg et al., 1994; Moeller et al., 1999), and later on whole brain voxel level (Asanuma et al., 2005; Asanuma et al., 2006; Ma et al., 2007). The pattern itself features positive relative deviation from mean subject and group values in the basal ganglia, thalamus, and cerebellum, as well as negative deviations in premotor and parieto-occipital cortex. In addition to providing biologically relevant information regarding the functional topography of the disease (Huang et al., 2007c; Lin et al., 2008; Hirano et al., 2008; Tang et al., 2010b), SSM also simultaneously quantifies the magnitude of the expression of this network for each subject (i.e., the PC scalar or subject score). This measure strongly correlates with independent clinical descriptors of disease severity (e.g., Eidelberg, 2009). Indeed, PDRP scores have been employed for purposes of differential diagnosis (Spetsieris et al., 2009; Tang et al., 2010b), for the assessment of disease progression (Huang et al., 2007c; Tang et al., 2010a), and in the evaluation of the efficacy of therapeutic interventions (e.g., Trost et al., 2006; Asanuma et al., 2006; Feigin et al., 2007a) for this disorder.

The facility of prospective evaluations and single subject testing is an advantage of this methodology over inferential methods that rely on statistical assessments of assumed correlations. Although the SSM model has been successfully applied to a large number of PET datasets including non-PD patient groups and other modalities such as SPECT and fMRI (Feigin et al., 2002; Eckert et al., 2007; Huang et al., 2007a) the full potential of this powerful method has not been realized extensively by other groups because of certain methodological ambiguities. The purpose of this paper was to address these issues and enable users to utilize the method successfully.

The precise methods of applying SSM to identify and validate specific disease patterns from patient and control scan data, and the interpretation of the resulting topographic changes, have not always been clear (Borghammer et al., 2008; 2009). For example, an essential initial step incorporated in the formulation serves to remove overall, major subject and group global effects, thereby revealing inherent small disease-related deviations in the underlying values. The decomposition of the spatial covariance of these deviations into linearly independent (orthogonal) principal components (PCs) allows the dimensionality of the data to be reduced into the single or limited set of spatial covariance patterns that can be attributed to the disease process. The corresponding eigenvectors comprise voxel weights of the covariance structure and not absolute physiological measures. Displayed as Z-scored maps of the voxel weights, they reflect the pattern of interconnectivity of deviant pathological areas.

We review here the basic mathematical formulation of SSM to elucidate essential concepts underlying this approach, as well as the nuances that may influence results. In particular, we

discuss the effects of global normalization, centering of the data, thresholding and masking constraints, and the criteria for the selection of significant principal components on pattern identification. To illustrate these concepts, we derived variants of the PDRP topography that is currently being used in our studies (Ma et al., 2007), assessing the impact of these methodological features on the derivation of disease-related spatial covariance patterns from an established population of PD patients and healthy controls. The expression of these variant patterns was computed prospectively in independent groups of clinically ascertained patients of varying disease duration. Based on forward analysis of the various patterns we demonstrate the robustness of this SSM approach and its suitability for the study of neurodegenerative disorders.

2. Methods

In this section, we describe data acquisition and demographics of our data sets, the basic formulation of SSM, as well as image processing and methodology issues.

2.1 Data Acquisition and Software

For this study we used FDG PET images, acquired on a GE Advance tomograph (Milwaukee, WI, USA) at North Shore University Hospital (Eckert et al., 2005). Images were transformed to a normalized Talairach-like space (MNI) using SPM99 [<http://www.fil.ion.ucl.ac.uk/spm>]. SSM analysis was performed on a PC, MATLAB (ver. 2006b)-based platform (Mathworks, Sherborn, MA) using customized neuroimaging software scanvp ver. 6.1 (Eidelberg et al., 1994); available at <http://www.feinsteinneuroscience.org/>. This toolbox incorporates MATLAB and C-language based processing and statistical routines.

2.2 Group Data

The subjects in this study belonged to three separate groups. Demographic details are presented in Table 1. The first (Group1) was the original group of 33 PD patients of moderate disease duration (9.3 ± 3.8 years) and 33 age-matched healthy subjects used to derive the previously validated PDRP network (Ma et al., 2007, Fig. 1). The second (Group 2) was comprised of 30 other PD patients who had shorter disease duration (2.2 ± 1.1 years) at the time of imaging and in whom the diagnosis was confirmed on long-term clinical assessment, as well as 30 age-matched normal controls. The third group (Group 3) was comprised of nine additional PD patients with advanced dementia (PDD) and long disease duration (14.6 ± 5.8 years), as well as nine age-matched normal subjects.

2.3 SSM/PCA Standard Formulation

Consider “raw” *resting state* functional brain data obtained from modalities such as PET (blood flow/metabolism), SPECT (blood flow), or fMRI (blood flow/oxygen-level dependent signal) from a combined group of patients and controls. The aim of PCA is to reduce dimensionality of datasets that involve a large number of interrelated variables (Rencher, 1995; Jolliffe, 2002). Essential features of the SSM model are its inherent preliminary log transformation and “double centering” of the data (i.e., subtraction of the mean data from both rows and columns of the subject by voxel data matrix) before applying PCA. This insures that results will be invariant to subject and regional scaling effects. As indicated by Jolliffe (2002), column centering is needed when there is substantial covariance across “observations”. Likewise, row centering is needed when there is substantial covariance between the various “measures” acquired in a given subject's data. Centering transforms the data into a coordinate system that relates differences from mean values. In the case of brain data, subjects and regions (voxels) are represented by the rows and columns of the data matrix. Log transformation of the images separates essentially meaningless

multiplicative scaling effects into additive components that are subsequently removed by the centering operation. Thus, these operations serve to normalize the data with respect to mean activity. The singular value decomposition (SVD) of the covariance matrix results in a set of independent, orthogonal principal components where most of the variance is accounted for in the first few components because variance is maximized and cross-terms are eliminated by the preliminary centering operations. Further, SSM retains only those PCs that are relevant to disease as judged by the discriminative accuracy of patient versus control pattern scores. In the following we summarize the basic steps of SSM in the analysis of resting state brain imaging data. The mathematical formulation is similar to the regional approach described by Moeller and Strother (1991) and Alexander and Moeller (1994), and the voxel-based approaches summarized in Spetsieris et al. (2006), Ma et al. (2007) and tutorially in Eidelberg (Eidelberg, 2009).

1. An initial smoothing and normalization to a common space (e.g. MNI), can be performed using SPM [<http://www.fil.ion.ucl.ac.uk/spm>] or other software so that there is a one-to-one correspondence of voxel values P_{sv} between subjects for every subject s (1,...,M) and every voxel v (1,...,N). (It should be noted that the voxel data parameters, e.g. GMR, given in this study are derived from raw PET count images in units of kBq/cc although, this detail would not affect outcome in any way. Also, with regard to notation, we have used italics s , v to distinguish variable values from specific values of s and v . Matrices are represented with capital bold letters and vectors in bold upper or lower case. Non-bolded parameters refer to scalar parameters, voxel values or image names). The voxel values of each subject's image are strung together to form a single row vector of the $(M \times N)$ data matrix **P**.
2. Brain data is masked to reduce low values and noise. This can be done by, e.g. *a*) using a predefined volume mask, or *b*) using a specified lower threshold to create individual masks that are multiplicatively combined to create one common mask that includes only non-zero values for all subjects.
3. The masked data is logarithmically transformed:

$$P_{sv} \rightarrow \text{Log } P_{sv}$$

4. Each subject's log data are centered with respect to its mean value LGMR_s . This results in the singly centered data values Q_{sv} ["row centered" (Joliffe, 2002)],

$$Q_{sv} = \text{Log } P_{sv} - \text{LGMR}_s, \\ \text{where } \text{LGMR}_s = \text{mean}_{\text{vox}} (\text{Log } P_{sv}) \quad (1)$$

Although the term GMR_s typically refers to the global metabolic rate, here it denotes a more general parameter, i.e., the mean across voxels of the brain data of subject s .

5. The row centered log data matrix **Q** is further (column) centered with respect to the combined *group* mean by subtracting the mean value across all subjects for each voxel v . The group mean over subjects is referred to as the Group Mean Profile (GMP) image and the resulting doubly centered data is defined as the Subject Residual Profile (**SRP**) matrix whose elements are:

$$\text{SRP}_{sv} = Q_{sv} - \text{GMP}_v, \\ \text{Where, } \text{GMP}_v = \text{mean}_{\text{sub}} (Q_{sv}) = \text{mean}_{\text{sub}} (\text{Log } P_{sv}) - \text{mean}_{\text{sub}} (\text{LGMR}_s). \quad (2)$$

Summarizing, $P_{sv} \rightarrow \text{Log } P_{sv} \rightarrow Q_{sv} \rightarrow \text{SRP}_{sv}$

6. In view of the above equations, we can describe the SRP_{sv} voxel values for each subject as the difference of the original masked data values in log space from the subject mean constant LGMR_s , and the voxel values for the group mean GMP_v image [similar to eqn 1 in (Alexander and Moeller, 1994), ignoring the error term]:

$$\text{SRP}_{sv} = \text{Log } P_{sv} - \text{LGMR}_s - \text{GMP}_v \quad (3)$$

7. For purposes of further discussion, this equation can also be written as:

$$\begin{aligned} \text{SRP}_{sv} &= \text{Log } P_{sv} - \text{mean}_{\text{sub}}(\text{Log } P_{sv}) - [\text{LGMR}_s - \text{mean}_{\text{sub}}(\text{LGMR}_s)] \\ \text{where,} \\ [\text{LGMR}_s - \text{mean}_{\text{sub}}(\text{LGMR}_s)] &\equiv cC_s \text{ (Subject Centering Constant)}. \end{aligned} \quad (4)$$

This expression emphasizes that the SRP voxels represent deviations of the subject log image values from the group mean log image values. The subject centering scalar cC_s corresponding to the difference of the subject global mean LGMR from the group mean LGMR value. We see, therefore, that as a result of column and row centering, SSM does not operate directly on voxel values but on log differences from mean subject and mean regional values.

8. The SVD of the *subject by subject* and *voxel by voxel* covariations of the **SRP** data to derive the SSM GIS patterns and associated subject scores is briefly described in Appendix A. As a result of the SVD analysis, the SRP voxel values for each subject can be expressed as a sum of the product of the derived orthogonal, unitized **GIS_k** ($k=1,2,\dots,M$) vector voxel weights multiplied by their corresponding subject score:

$$\text{SRP}_{sv} = \sum_k \text{Score}_{ks} \text{GIS}_{kv} \quad \text{or} \quad \text{SRP}_s = \sum_k \text{Score}_{ks} \text{GIS}_k \quad (5)$$

9. To be considered as a disease-related pattern, a single or linear combination of GIS vectors must have associated subject score values that separate the patient and control groups at a pre-specified statistical threshold, typically $p \leq 0.001$ (two-sample Student's *t*-test). Of note, several statistical methods (discussed below) have been suggested to determine the optimal number of PCs to retain for purposes of delineating a “disease-related” pattern. Only the first few GIS vectors, corresponding to different major sources of the total spatial variance in the SRP data, are usually meaningful. It should also be noted that both the GIS vector and its negative form are mathematically equivalent solutions of the eigenvector equation with a corresponding sign flip of associated subject scores. Only the GIS form corresponding to positive mean patient scores relative to normal subject scores is considered to be relevant.
10. Because of the orthogonality of the GIS vectors, we can evaluate the subject score for the k th GIS or any linear combination of GIS vectors as the inner dot product of the subject SRP row vector and the GIS vector:

$$\text{SRP}_s^T \cdot \text{GIS}_k = \left(\sum_k \text{Score}_{ks} \text{GIS}_k^T \right) \cdot \text{GIS}_k = \text{Score}_{ks} \quad (6)$$

Fortuitously, this formula can be used in forward application to compute pattern scores for prospective subjects on an individual basis. (In early studies we termed this operation “topographic profile rating” (TPR) (see e.g., Eidelberg et al., 1995; Moeller and Eidelberg, 1997; Spetsieris et al., 2006). In the computation of TPR scores, subject SRP voxels are evaluated using eqn.3, where we can use the previously determined reference population GMP image (considered to be a practically invariable group characteristic) or recalibrate its value using a similar new population of reference patients and controls. The immediate applicability of the SSM patterns in prospective data to test individual results or the replicability of group findings is one of the salient strengths of this methodology.

GIS vectors are usually depicted in Z-score format representing voxel maps of standard deviations above and below the mean voxel weight. As such, the SSM GIS regional voxel weights do not represent absolute measurements of cerebral metabolism or blood flow. Rather, the voxel loadings on a given spatial covariance pattern reflect deviations in regional brain function from corresponding component mean values derived from the combined normal and patient dataset. The corresponding subject score values can be examined using standard hypothesis testing approaches to determine if there are significant score differences between patient and normal subjects for each GIS. A GIS pattern whose scores provide highly significant group discrimination (i.e., low p-values) is considered to represent a disease-related spatial covariance pattern, i.e. in a map of inter-related brain regions whose function is abnormally expressed in patients. In Z-scored GIS representations there will always be positive and negative values with respect to the mean, with gradations interpreted by the pseudocolor stripe. The pattern therefore can be interpreted as an invariate characteristic of the group where its expression in individual cases is represented by the value of the associated subject score. In more severe patients, the corresponding score may be greater so that the positive areas represent greater SRP component deviations from subject mean and group values. Fig. 1 depicts an established Parkinson's disease-related network (PDRP) (Ma et al., 2007), $p=1.94e^{-12}$, where in MATLAB notation the exponent base e corresponds to the number 10. In the following section, we tested the robustness of the PDRP biomarker and the SSM/PCA method by evaluating the consequence of various modified methods of network derivation.

3. Practical Computational Issues

Several common and some unconventional computational issues have surfaced in discussions of the application of SSM in functional imaging data for disease cohorts (Petersson et al., 1999; Ma et al., 2009; Borghammer et al., 2008; 2009; 2010):

1. How do we best determine a group mask or threshold?
2. How many principal components should be included in GIS network derivation?
3. What is the effect of pre-normalization by global scaling on the analysis?
4. How would normalization using the cerebellum or other regional reference regions affect results?
5. What is the effect of log transformation on the analysis?
6. What is the effect of data centering on the analysis?
7. How do overactive (“red”) areas relate to underactive (“blue”) areas within a particular GIS pattern?

In this study, we address these questions using simple mathematical proofs for the more obvious concerns and use empirical evidence to investigate the more complex issues.

3.1 Thresholding and Masking Constraints

Clearly, the GIS patterns in SSM refer only to the voxels within the boundaries imposed by the threshold or an external mask. Therefore, low value voxels, noise-related artifacts, and areas not involving brain activity (e.g. eyes), should be removed by pre-processing software. If a threshold is specified, it is initially applied to each subject to create separate 0/1 masks that are multiplicatively combined to create a single mask of common non-zero voxels for the entire group. The ideal threshold may be application dependent. We include a default in our software that evaluates the lower threshold as the value that is 35% of the whole brain maximum. This can be overwritten if we want to evaluate lower- or higher-value voxels. Alternately, a pre-derived mask can be used (e.g. a gray matter mask) or, in specialized applications, we can apply a mask that focuses on certain areas of interest (see Section 4) (Spetsieris et al., 2009). The use of a lower threshold would allow a larger area of low-value voxels to be assessed. This, however, may also result in additional noise and/or the introduction of global factors of little relevance to the disease effects. Preprocessing methods described in the literature are to a large extent based on empirical criteria (e.g., Friston et al., 1995; Gwady et al., 2001; Habeck et al., 2008). Prior anatomical knowledge of significant regions is not included (except in special applications) because SSM is a non-inferential, exploratory method. SSM can be applied following an initial univariate statistical determination of thresholds such as F-test based masking used in SPM. However, it should be noted that this can compromise its ability to detect covariant components that may not be discernible by the GLM (Friston et al., 1995; Petersson et al., 1999) since in univariate analysis disease-related effects are not distinguishable from other, potentially overshadowing, sources of variance. PCA attempts to reduce the dimensionality of the data by capturing the main sources of variance in a limited number of components (Rencher, 1995; Joliffe, 2002). Plausibly, the ideal threshold would result in the smallest number of significant GIS vectors (preferably only one), the greatest effect in the data (largest eigenvalue and therefore largest variance contribution) and provide best between-group separation (lowest p-value).

We tested the effect of threshold variation on the discrimination of patients from controls in the analysis of the Group 1 data. This was accomplished by assessing the corresponding variation in the p value of the most significant PC in this SSM/PCA analysis, i.e., PC1 (GIS 1). The results shown in Fig. 2 demonstrate an optimum value ($p = 1.9e^{-12}$) at 0.35 of the global maximum (This value was originally selected to define the PDRP). At lower thresholds, disease effects are distributed in additional PCs that can also include extraneous sources of variation. To compare networks and scores calculated from the same voxels, we applied the ($T = .35$ of max) PD33 mask derived in the original generic derivation of the PDRP in all of the following analyses. In future software distributions, a default grey-matter mask will be provided to simplify this step in whole-brain analysis.

3.2 Criteria for Network Selection

How many PCA factors should be retained in network derivation is a common question in SSM analysis. Indeed, the following procedures have been applied, both singly and in combination.

- a. Select only the single most prominent (discriminating) component?
- b. Combine components whose individual p values are less than a fixed value?
- c. Use the Akaike information criterion (AIC) (Akaike, 1974) to compare the accuracy of group discrimination using different numbers of PCs and select the combination with the lowest AIC value (e.g., Habeck et al., 2008).
- d. Employ Scree-Cattell test (Joliffe, 2002; Cattell, 1966).

- e. Combine successive components so that the overall variance is less than a fixed percent and then apply an additional selection criterion. Empirically, we have found adequate the simple rule of retaining components corresponding to a cumulative percent of variance that does not exceed about 50%. This amounts to limiting pattern derivation to the portion of the data containing the most relevant subject region effects. Within this subspace we retain only those components with individual significance levels below a pre-specified threshold (e.g., $p < 0.05$). As noted, this is an empirically derived approximate cutoff. In practice the vaf and p-values of borderline components can be taken into consideration. Since SSM is a non-inferential method, prior neurobiologically based knowledge is not overtly considered but the visual representation of the borderline patterns and subject scores may have a limited subjective influence.

The selected components are then combined into a single GIS vector for further analysis. The optimal coefficients for the linear combination of the selected PCs can be determined by logistic regression of the corresponding subject scores (Hastie et al., 2001). In this study we used MATLAB's *gmlfit* function that provides a generalized linear model for a binomial distribution with a 'logit' link. In rigorous follow-up we can perform bootstrapping procedures to test the robustness of the pattern (Habeck et al., 2008). The analysis can be repeated by resampling the original dataset in multiple permutations to evaluate corrected p-values. Further, assuming that discriminative criteria are considered the decisive factor in component inclusion, we can perform k-fold cross validation with prospective data, with and without potential borderline PCs, to compare predictive accuracy (Spetsieris et al., 2010).

Of note, the discriminant capacity of the resulting network may be increased in the derivation dataset by including additional components. Nonetheless, this does not necessarily translate into increased discrimination in the test data. These additional sources of variation may correspond to a small subset of patients, outliers or inadvertent error factors or noise and, therefore, careful additional testing is necessary for validation. Moreover, greater predictive accuracy may not necessarily imply superior representation of the covariance structure because the logistic model can place undue weight in the inclusion of minor sources of variance.

In the derivation of the composite PD33 GIS using Group 1 data masked at $T=.35$ (Fig. 3), the PDRP (GIS 1) accounts for about 21% of the variance. Over 55% of the variance is accounted for by PCs 1 to 7 even though PCs 2 to 6 do not discriminate patients from normals. The result of adding an additional small source of variation PC 8 (vaf 2.61%, $p=0.004$), is displayed in Fig. 3 (detailed in suppl. Table 1). In the derivation Group 1, the component p values for PC 1, PC 8 and the combined PC1, 8 were $1.9e^{-12}$, 0.004, and $2.4e^{-16}$, respectively. However, these values were $4.8e^{-7}$, .668, and $2.1e^{-7}$ in the prospective test Group 2 and $1e^{-4}$, .387, and $9.7e^{-6}$ in test Group 3. We see that, although the additional component does not discriminate patients from normals in either prospective group, its inclusion in the composite PC 1, 8 can lead to paradoxically better discrimination than PC 1 alone.

We note though, that including lesser sources of variance (i.e. small eigenvalues) in a logistic model can lead to erroneous results and must be interpreted with caution (Hastie et al., 2001). Logistic regression does not take into account the relative weight of the vaf values. A small vaf may represent an outlier or error source component or a physiological factor that has not manifested itself fully in all subjects or that innately only minimally effects the measured parametric variable. However, the logistic model does not consider vaf values in weighting components and may magnify the signal in order to obtain optimal discrimination. The resulting composite GIS may correspond to lower p-values of patient/normal score discrimination but may be misleading in terms of the relative significance of

the GIS voxel weights within the overall covariance structure of the data's measured parameter. For this reason we avoid the inclusion of low var value PCs. Thus, linear addition using logistic regression that allotted PC₈ high (.4) coefficient weight in determining the PC₁₈ resultant GIS, may result in networks with improved *discrimination* that do not necessarily topographically depict major sources of variation in disease. A Bonferonni correction weighted by the relative component eigenvalue (Abdi, 2007) may be of use in these cases but was not considered here because of the relative non-significance of the var value. That said, the inclusion of additional PCs associated with higher eigenvalues and, therefore, larger variance contribution is often necessary as is seen in examples below.

3.3 Global Normalization in SSM of Log Transformed Data?

The subject of global normalization is an important issue that has been addressed in various applications of functional neuroimaging (Alexander and Moeller, 1994; Friston et al., 1995; Petersson et al., 1999; Ma et al., 2009). SSM has a built in simple and effective normalization process that has been recently made controversial because of misconceptions regarding its basic use and effect (Borghammer et al., 2008; 2009). As is implied in its name, the SSM model examines the structure of the data removed from any global scaling confounds. In global mean normalization, the mean of subject values (GMR) is evaluated over all voxels within a masked or thresholded space and used to divide the data. This form of scaling is used routinely in mass univariate analysis to help discriminate patients from normals based on deviations of ratio norms. However, disease effects may involve subtle deviations from normal values that may not be discerned using ratio scaling alone. Thus, subject means may remain fairly normal in parkinsonism, obscuring small underlying deviations. As shown below, the SSM approach effectively removes mean values prior to analysis by requiring log transformation of the data before subtraction of the mean. Thus, pre-processing by normalization of a subject's values by scalar division, whether with respect to the global mean or another measure (e.g. the mean of a "neutral" or other brain region such as the cerebellum), does not have any further effect on standard SSM analysis. The proof is trivial but is included here because it has been a topic of misunderstanding in the past (Borghammer et al., 2008; 2009).

Proof—Consider a subject's data values P_{sv} . As described above, SSM initially evaluates the log of each data value and subtracts the subject mean log value over all voxels v in the data space (i.e., it centers the data with respect to the subject mean):

$$Q_{sv} = \text{Log } P_{sv} - \text{mean}_{\text{vox}} (\text{Log } P_{sv}).$$

If we pre-normalize by a scaling constant C_s (e.g. $1/\text{GMR}_s$), the P_{sv} voxel values are replaced by values $C_s P_{sv}$. The subject centered values become:

$$Q_{sv} = \text{Log } (C_s P_{sv}) - \text{mean}_{\text{vox}} (\text{Log } (C_s P_{sv})).$$

However, this expression reduces to the same value as before because $\text{Log } C_s$ and the voxel mean of $\text{Log } C_s$ are the same:

$$\begin{aligned} Q_{sv} &= \text{Log } C_s + \text{Log } P_{sv} - \text{mean}_{\text{vox}} (\text{Log } C_s + \text{Log } P_{sv}) \\ &= \text{Log } P_{sv} - \text{mean}_{\text{vox}} (\text{Log } P_{sv}) + \text{Log } C_s - \text{mean}_{\text{vox}} (\text{Log } C_s) \\ &= \text{Log } P_{sv} - \text{mean}_{\text{vox}} (\text{Log } P_{sv}). \end{aligned}$$

Thus, the effect of scalar normalization is removed for each subject in the initial step of the SSM calculation. Indeed, the built in removal of the mean subject logarithmic value effectively globally normalizes the data to a common scale without requiring a separate pre-processing step. The rest of the analysis involves group (column) centering and is unaffected because the evaluation is performed on the Q_{SV} data.¹ We verified the pointlessness of pre-scalar normalization in log transformed data by performing SSM analysis with and without scalar normalization. As expected, we obtained exactly the same result as in Fig. 1.

In summary, SSM operates on the underlying data covariant interactions once any multiplicative global effects have been removed, which is a distinct advantage of the logarithmic operator (Joliffe, 2002). While one can perform a modified SSM without logarithmic transformation, in such cases the data need to be globally pre-normalized. That said, the choice of normalization constant can then influence the PCA results. To illustrate this point, we performed non-log modified SSM analysis on the Group 1 data using *a*) no pre-scaling, *b*) ratio normalization with GMR, and *c*) mean cerebellar normalization. We compared the results of these analyses with those of the standard log-transformed SSM analysis.

3.3.1 Non-Log PCA – No Pre-Scaling—In this analysis, Group 1 data was processed without log transformation. To conform to routine procedure, the images were not pre-scaled and we utilized the same PD33 mask that was applied in the original PDRP derivation (see above). In contrast to the standard SSM where only one significant PC was obtained, the modified analysis produced a large set of PCs that did not discriminate as well individually. That said, five of the first six components separated patients from controls with $p < 0.04$ (Fig. 4, suppl. Table 2), with improved discrimination when combined.

Using logistic regression, we combined the first three PCs (PC_{1-3}) accounting for 53% of the variance and up to the first six PCs (PC_{1-6}) totaling 66% of the variance. The correlation between voxel weights on any PC image vector (or combination of image vectors) resulting from modified SSM analysis and those on the PDRP (i.e., PC_1 of the unmodified log-transformed analysis) was calculated using a customized MATLAB based routine as the square of the Pearson's linear correlation coefficient (R^2) between all the corresponding non-zero voxel values. The combination of multiple factors produced patterns that was highly correlated with the PDRP ($R^2 = 0.75$ for PC_{1235}). The addition of the small variance PCs 5-6 increases the accuracy of group separation in both the derivation set (Group 1) and the testing sets (Group 2 and Group 3). These components did not, however, correlate with the log analysis component PC_8 ($R^2 = 0.0004$). Indeed, the validity of such minor sources of variance needs to be carefully evaluated in additional prospective cohorts of patients and controls. Overall, the minimum composite p value ($p(33) = 4.4e^{-16}$, $p(30) = 2.1e^{-7}$) was found to be very close to those obtained using log transformed data (suppl. Table 1) ($p(33) = 2.4e^{-16}$, $p(30) = 2.1e^{-7}$). We note, though, that in the standard analysis, most of the

¹We thank one of the anonymous reviewers for supplying the following alternative proof: Imagine for simplicity we just have three regions, and the signal values in these regions are denoted as alpha, beta, gamma. Scaling by the geometric mean (which is equivalent to subtracting the arithmetic mean of the log signal values for the three regions from the subject's log image) equals division by the factor:

$F = (\alpha * \beta * \gamma)^{1/3}$, using MATLAB symbols for multiplication (*) and root (^) operations.

One can easily see that division by this factor is invariant with respect to arbitrary referencing. For instance, if we use region 1 as reference we obtain:

$F' = (\alpha/\alpha) * (\beta/\alpha) * (\gamma/\alpha)^{1/3} = (\alpha * \beta * \gamma)^{1/3} * (1/\alpha)$
 $= F * (1/\alpha)$

The referencing to region 1 thus imposes an additional overall factor alpha, which compensates for the division by alpha, so that in the end each region's signal is unaffected:

$\text{signal}' / F' = \alpha * (\text{signal}/\alpha) / F = \text{signal} / F$.

These considerations easily generalize to arbitrary numbers of regions.

disease-related variance was captured in a single GIS. Without the logarithmic transformation, this is distributed among numerous PC fragments that are likely to include non-relevant effects.

3.3.2. Non-Log PCA – With Global Mean Pre-Scaling—We performed non-log modified SSM/PCA on the Group 1 derivation data following ratio pre-scaling with global mean (GMR) values. The resulting analysis revealed two components (termed gPC₁ and gPC₈) with $p < 0.01$; these patterns were topographically very similar to those derived in the standard analysis (Fig. 5). Voxel-wise correlations with the PDRP and TPR discriminant p values are reported in Table 2. We see that with global normalization the derived PC₁ vector is almost identical to the PDRP ($R^2 > 0.98$) and corresponds to essentially equal p values in the derivation data. TPR scores for the gPC₁ pattern computed prospectively in the globally normalized data from Group 2 separated patients and controls with less accuracy ($p = 2e^{-4}$, higher p -value) than the corresponding PDRP values for log-transformed data ($p = 4.8e^{-7}$).

3.3.3. Non-Log PCA – With Cerebellar Pre-Scaling—To dismiss the theory that pre-scaling with an appropriate “neutral” region is necessary to avoid assumed “artifacts” induced by standard SSM (Borghammer et al., 2009), we performed non-log PCA with cerebellar (CER) pre-scaling. Note, in fact that the relative increased covariant weighting of the cerebellum in SSM derived disease patterns is considered to be artifactual by this account which assumes the cerebellum to be an invariant region.

We used a predefined template of left/right cerebellar (CER) regions-of-interest (ROIs) in the lower axial planes ($Z = -44\text{mm}$ to $Z = -30\text{mm}$) to calculate mean metabolic values for this reference region. We performed non-log modified SSM analysis on the Group 1 data where each image was divided by its corresponding CER value. The resulting PCA revealed two PCs (termed cPC₁ and cPC₈) which accurately separated the two groups ($p < 0.004$). Voxel weights on these patterns were highly correlated with the corresponding PCs derived using standard log-transformed SSM and GMR pre-scaled data (Fig. 5, Table 2). TPR values were calculated for Group 2 data (CER pre-scaled in the same manner). Although for cPC₁, p values were slightly lower (i.e., more discriminating) in Group 1 compared with gPC₁ and PDRP, subject scores for this pattern had the highest p value (i.e., least discriminating) p -value in the prospective Group 2. Indeed, in the prospective Group 2 data, the PDRP provided consistently better group separation than the corresponding patterns derived from the Group 1 data using either global or cerebellum ratio pre-scaling. Both gPC₁ and cPC₁ voxels and corresponding subject scores were highly correlated with each other and with the PDRP ($R^2 \geq 0.97$, Table 3). Correlation with PC₁₋₃ (no pre-scaling) was not as strong.

For all cases, the single PC₈ component did not discriminate patients from normals in the prospective groups. The non-log PC₈ components for GMR and CER scaling were highly correlated with the log analysis PC₈ ($R^2 = 0.8621, 0.9200$).

3.3.4 Re-Derivation of GIS Patterns Using Standard SSM and Non-Log Pre-Scaling—We performed a new SSM GIS derivation analysis on the prospective Group 2 to compare the correlation of newly derived GIS patterns with those derived in Group 1. The predefined PD33 mask was used to insure voxel by voxel correspondence although the results with a similarly generated Group 2 mask (not reported here) did not differ substantially. We performed a standard log-transformed data analysis as well as non-log GMR and CER ratio pre-scaled data analyses. The PCA generated two significant PCs for the standard and GMR pre-scaled methods and three significant PCs for the CER scaled data (Fig. 6 and suppl. Tables 3-5).

We see (suppl. Table 4) that the composite PC_{12} derived with the standard log SSM in Group 2 is highly correlated with that derived with GMR normalized data (vox $R^2 = 0.97$) and somewhat less correlated with that derived with CER prescaled data (vox $R^2 = 0.90$). Correlation with the PDRP (vox $R^2 = .80$) was also high considering that the two groups involved two independent groups of patients at very different stages of disease progression. Subject score correlations with prospectively computed PDRP values in Group 2 were high in all cases ($0.84 \leq R^2 \leq 0.95$). The composite component under log transformation (PC_{12}) separated patients from controls in Group 2 with accuracy comparable to PDRP TPR values. Moreover, its TPR value computed in Group 1 data ($p = 1.04e^{-12}$) compared favorably with PDRP values in these subjects ($p = 1.9e^{-12}$). Indeed, PC_{12} achieved superior group separation (lowest p-value) in prospective analysis of Group 1 data compared with patterns derived by modified approaches in the Group 2 data. For non log transformation the lowest p values were achieved for the composite gPC_{12} for GMR normalized data ($p = 2.13e^{-7}$). A comparable value was obtained for CER normalized data ($p = 2.2e^{-7}$) when the third component was included. The third component did not accurately separate the prospective Group 1 patient and control groups ($p = 0.26$) but improved discrimination when combined with the first two components ($p = 3.8e^{-12}$, suppl. Table 3). That said, this pattern did not discriminate Group 3 patients from normals ($p = 0.476$) and did not improve discrimination when combined with the first two patterns. Moreover, this pattern did not correlate with the PDRP and its significance could not be interpreted.

In some of the above non-log derivations the discriminant power of the resultant PCs was found in some cases to increase slightly. Although the correlation with the log based result usually remained strong with similar discriminant accuracy, the involvement of additional PCs can complicate the interpretation of the findings. The composite GIS patterns in Group 2 were able prospectively to discriminate the Group 1 data with greater accuracy than in the derivation Group 2 cohort, achieving comparable separation to values derived directly in Group 1. Clearly, discriminant performance is a measure of both the disease pattern and the characteristic contrast between patient and controls of the group in which it is tested. Thus, Group 1, which included patients with longer disease duration, was associated with lower p values regardless of whether the disease pattern was derived in Group 1 or in Group 2.

3.4 Mean Centering?

Centering of the data with both the subject mean and the group mean is a key initial processing step in SSM (Alexander and Moeller, 1994; Petersson et al., 1999) steady-state data. The importance of centering in PCA has been historically a complex and controversial issue that has also been addressed in a wide range of unrelated fields of study, most notably in atmospheric predictions of global warming (Jolliffe, 2002; Miranda et al., 2008; Cadima and Jolliffe, 2009). In dynamic studies, centering is controversial because it can inadvertently remove essential time dependent effects (Gwady, 2001). However, in the study of steady-state disease effects, group mean centering is intuitively necessary since it allows us to look at differences from group norms. Row centering reduces subject global effects that may be due, e.g. to differences in dosage or measuring parameters. We have attempted here to investigate more fully the consequences of subject data de-centering in the context of SSM/PCA analysis.

3.4.1 Uncentered Subject Log Data Analysis—In a previous section we demonstrated that the removal of the mean of the subject log data insured the removal of any preexisting subject scaling factor. To further illustrate the value of centering log data with respect to the subject mean, we performed SSM analysis on the Group 1 derivation dataset without removal of the subject mean log value. The resulting PCA decomposition illustrated the occurrence of a strong global factor that could not discriminate patients from controls but

accounted for most of the variance (97%). However, the same network GIS, previously shown to exhibit a strong disease association, appeared essentially unaltered as GIS 2, now corresponding to only a small percentage (less than 1%) of the overall variance, which can be easily overlooked (Fig. 7).

The voxel correlation of the PDRP with GIS 2 was $R^2 = 0.9997$ ($p < 0.0001$), whereas with GIS 1 there was no correlation ($R^2 = 0.0126$). The corresponding correlation between PDRP and GIS 2 subject scores was equally high ($R^2 > 0.998$, $p < 0.0001$), whereas with GIS 1 there was no correlation ($R^2 = 0.002$, $p = 0.74$). By contrast, there was a near perfect correlation of the log data GMR with GIS 1 Scores ($R^2 \approx 1.0$, $p < 0.0001$) (Fig. 8a) and no correlation with GIS 2 scores ($R^2 < 0.0001$, $p = 0.999$) (Fig. 8b). Thus, the subject centering of the log data serves to remove the major source of variance stemming from the global mean values, which do not discriminate patients from controls (p values using GIS 1 Scores and log data GMR were 0.4461 and 0.4458, respectively).

3.4.2. Cerebellar (De-)Centered Log Data Analysis—We tested the effect of attempting to center the subject data with respect to a “neutral” brain region such as the cerebellum, which has been suggested as an alternative to GMR in the setting of widespread reductions in cortical metabolic activity (Borghammer et al., 2009; 2010). This approach decenters **SRPs** with respect to global values by subtracting the mean log cerebellar value of the subject, as opposed to the mean log value for the entire brain. Bearing in mind that substantial controversy exists concerning the mathematical validity and interpretation of decentered PCA (Gwady et al., 2001; Jolliffe, 2002; Cadima and Jolliffe, 2009), we evaluated this form of normalization empirically in the combined PD and normal scan data. In this case the subject centering constants in eqn.4 becomes:

$$cC_s = CER_s - \text{mean}_{\text{sub}}(CER_s), \quad \text{where } CER_s = \text{mean}_{\text{CER}}(\text{Log } P_{sv}).$$

We calculated the modified subject centering constants by log transforming the masked data and evaluating the mean of the voxels within the top 10% of the three dimensional region maxima. Left/right values were averaged and the mean centering constant for each subject was evaluated. PCA analysis was performed on the log data values once the mean group log image and the centering constants were removed.

Cerebellar mean decentering exerted a fragmenting effect on the PCA output (Fig. 9). Most of the variance was represented by the first component PC (46.6% vaf), which had relatively low group discrimination. By contrast, the second PC closely resembled the PDRP. The linear addition of PC₁ and PC₂ using logistic regression yielded PC₁₋₂, which correlated significantly with the reference PDRP in both voxel values and corresponding subject scores ($R^2 = 0.99$). The details of this analysis are given in suppl. Table 6. Interestingly, as a result of the decentering of the data, PC₁ and PC₂ were no longer independent ($R^2 = 0.45$), as can occur when PCA is applied to decentered data (Cadima and Jolliffe, 2009). Subtraction of the log of the mean cerebellar value (rather than the mean of the log data from this region) had a similar fragmenting effect (not shown).

4. SSM Findings in Parkinson's Disease

In longitudinal FDG PET studies, we have examined the effects of disease progression on PDRP expression (Huang et al., 2007c; Eidelberg, 2009; Tang et al., 2010a). These datasets have also provided us with an opportunity to examine the proposition that PD is associated with significant reductions in GMR even in early stages, and that “red” (positive) PDRP regions represent an artifact of extensive cortical hypometabolism (see e.g., Borghammer et

al., 2010). Although we have addressed this issue in an earlier paper (Ma et al., 2009), further computation evidence is now provided to clarify these matters.

4.1. Are there GMR reductions in PD?

GMR values were measured in several groups of subjects including early stage PD patients with no evidence of mild cognitive impairment (MCI(-), $n=19$), more advanced PD patients with single and multiple domain cognitive impairment (MCI(s), $n=21$; MCI(m), $n=28$), and patients with neuropsychological evidence of dementia (PDD, $n=9$) (Fig. 10a), (Huang et al., 2008; Eidelberg, 2009). The data revealed no GMR difference in the direct contrast of all PD patients ($n=77$; $p=0.27$; Student's t -test) with normal controls ($n=40$), or across the PD subgroups and the normal reference group ($F_{(4,112)}=1.75$, $p=0.14$; one-way ANOVA). Indeed, we found it informative to plot the GMR values alongside the PDRP scores as a function of disease duration (Fig. 10b). [Subject scores for the PD cognition-related metabolic pattern (PDCP, Huang et al., 2007b) are included for completeness.] This analysis disclosed a linear increase in PDRP expression with advancing disease duration ($r=0.969$, $p=0.001$); likewise, PDCP progressed linearly ($r=0.985$, $p<0.001$) over time, but at a slower rate ($p<0.02$, interaction effect) than PDRP. In this population, there was no correlation between GMR and symptom duration ($r=-0.267$, $p=0.610$). Interestingly, GMR reductions in PD patients were not significant relative to controls (-19.85% , $p=0.21$ Student's t -test) after 15 years of illness.

4.2. Does low GMR induce positive voxel weight artifacts in the PDRP pattern?

In other words, can positive (“red”) PDRP areas be interpreted as spurious findings, induced by global normalization to balance “blue” cortical deficits? To determine whether this is indeed the case, we derived an SSM pattern using the nine PDD patients (described above) and nine age-matched normal subjects (Group 3 in Table 1). We computed group separation p -values for subject scores on the resulting pattern, as well as from components of the pattern derived from corresponding red and blue areas. We found that this pattern (PDD_RP) was visually similar (Fig. 11) to the previously derived patterns for Group 1 (moderate cases) and Group 2 (early cases), even though it had been derived from a very small sample of patients with advanced disease. Both the voxel correlations with the PDRP and the Group 2 GIS₁₋₂ were high ($R^2 \geq 0.80$) as were score correlations ($R^2=0.98$).

It can be shown that scores for the complete pattern are a linear combination of the red and blue component scores. (For Group 3, $\text{Score_PC}_{12} = .568\text{Red_Score} + .569\text{Blue_Score}$.) We found (Table 4) that the red and blue component patterns of the PDD GIS (PC₁₂) were able separately to discriminate between Group 3 patient and control subjects as well as Group 1 and 2 subjects on a prospective single case basis (Fig. 12). Indeed, discrimination was accurate even though the blue and red voxels were topographically unrelated and derived from low GMR patients. In the PDD group, subject scores for the PDD GIS correlated closely with the corresponding TPR scores for the whole pattern as well as the red and blue components of the Group 1 PDRP and the Group 2 PC₁₂ pattern ($R^2 > 0.97$). In all groups, discrimination of patients from controls was relatively more accurate (lower p -values) for the red pattern compared to the blue and whole patterns, as evident in both direct and prospective assessments. The TPR p -values of Group 3 subjects for Group 1 PDRP scores were (whole: $1.1e^{-4}$, red: $1e^{-4}$, blue: $2e^{-4}$) and for Group 2 PC₁₂ scores (whole: $3e^{-4}$, red: $2e^{-4}$, blue: $6e^{-4}$). In fact, correlation of Group 3 scores derived for component patterns regardless of color was high in all three groups, with a minimum correlation magnitude of $R^2=0.94$ for the Group 3 red pattern scores and Group 2 blue pattern scores. Thus, it is evident that the effects of PD are expressed in both “red” and “blue” networks, and that the former quantify the disease process at least as well as the latter.

PDD_RP scores did not correlate with GMR values for any of the component patterns ($R^2 < 1$, $p > 0.2$). The strongest correlation was a negative relationship between GMR values and blue pattern scores ($R^2=0.1$, $p=0.2$) in Group 3. The p -values for discrimination between PDD subjects and normals based on GMR indicated inferior discrimination between patients and normals (e.g., 0.32 for Group 3, suppl. Table 7) relative to discrimination based on pattern scores, whether computed directly or by separate TPR of the whole PDD_RP, or of the red or blue components of this pattern.

4.3. Can the “red” pattern be derived directly within its own voxel space?

In a previous study we demonstrated that because of spatially widespread effects in neurodegenerative diseases, differential patterns that can effectively discriminate patients from controls can be derived in limited subsets of the original data space (Spetsieris et al., 2009). To address the possibility that the “blue” PD network regions define the covariance structure of the “red” pattern, we used the red areas of the PDD_RP pattern to specify a unique “red-only” mask for SSM pattern derivation. The new pattern (a combination of components 2 and 3) (Fig. 13) was very similar to the previous red component pattern (vox $R^2=0.82$, score $R^2=0.75$) that was derived directly from the PDD_RP, with significant discrimination of patients and controls ($p=8e^{-4}$). The high correlation of the two patterns indicates that the main source of covariation in the original red pattern is due to the red area voxels and that blue areas have only a limited effect. As expected by the z-transformation of the voxel weights (which is part of the SSM algorithm), the new pattern included regions with negative values relative to the red area mean GIS weight. However, this pattern was visually similar when displayed with a graded “red-only” color bar.

5. Discussion

In this technical note, we attempted to clarify several of the more challenging questions that arise in the application of SSM analysis in the study of brain disease. SSM incorporates simple yet powerful preprocessing in its logarithmic and double centering operations. The logarithmic operation separates irrelevant subject and regional scaling effects that are then efficiently removed by subject and group centering. In this way, the analysis specifically focuses on relevant *subject-by-voxel* interaction effects. As expressed by Joliffe (2002) and others (Kazmierczak, 1985) the results of such an analysis are invariant to different weightings of observations and to different scalings of the variables. We demonstrate that SSM spatial covariance patterns are unaffected by ratio normalization preprocessing using global or regional/cluster values. Nonetheless, the same cannot be said of mass univariate approaches (Borghammer et al., 2008; 2009; 2010). The overall metabolic deficits detected by F-test based masking and ratio-scaling methods without PCA may mask important underlying covariance networks. The GIS patterns represent “small signal” processes because they are derived from small data differences after larger subject and group mean effects have been removed. The basic invariability and specificity of the PD-related metabolic pattern across different stages indicates that this covariance structure is a true descriptor of the abnormal regional interactions that characterize this disorder. That SSM and mass univariate approaches are essentially different is evident from the observation that SSM patterns remain basically unchanged with advancing disease, whereas regional differences can vary considerably over time (Huang et al., 2007c; Habeck et al., 2008; Tang et al., 2010a). In this regard, disease progression is associated with an increase in SSM pattern expression whereas in univariate approaches this is likely reflected in a change in the regional profile itself. It is noteworthy that SSM does not depend on prior assumptions regarding the functional intercorrelations between brain regions. The spatial patterns detected in the analysis are entirely data-driven, reflecting relative contributions of all voxels within the covariance network and are not limited to isolated clusters or “blobs”. An

advantage of this methodology is that these patterns are invariant in prospective cohorts; their scalar expression in individual subjects can be directly utilized to test the model and to quantify rates of disease progression (Huang et al., 2007c; Feigin et al., 2007b; Tang et al., 2010a) and treatment effects (e.g., Asanuma et al., 2006; Hirano et al., 2008; Feigin et al., 2007a).

SSM pattern images are usually displayed as z-scored values that are representations of the number of standard deviations of each voxel above or below the mean GIS voxel weight. Thus, by definition, they will *always* depict areas of values above and below the mean. The higher voxel weights represent strongly covarying regions with relatively greater metabolic activity *within the network* (i.e., relative increases in network-related metabolism but not necessarily elevated metabolism in the absolute sense. Less significantly covarying voxels may not be reflected even if they encompass a larger overall area. In an analysis of a disease group that exhibits extensive hypometabolism, any derived GIS would still exhibit both high and low relative Z-scored weights. The data presented here indicate that the assertion that the positive (“red”) voxels identified in SSM analysis are artifactual is unjustified as is the assumption that they result from GMR normalization (Borghammer et al., 2008; 2010). We have shown here that GMR scaling or any other ratio normalization approach has no effect on standard SSM analysis of log-transformed data. Moreover, even were one to omit the log-transformation, the patterns resulting from the different scaling methods are quite similar. Further, for uncentered log-transformed data, which is equivalent to no global normalization in subject log space (i.e. no pre-scaling and no removal of the subject mean), the disease pattern emerges unscathed. It is, however, shifted in order of importance (i.e. to a lower eigenvalue) because of the dominant non-disease related global mean effect. Thus in SSM, the initial log transformation and centering processes serve to optimize the detection of disease effects within the reduced data dimensions of a single relevant PC pattern and associated subject scores.

Moreover, it is implausible that the PDRP and related covariance patterns would be sufficiently robust to be used for the accurate classification of single cases (e.g., Tang et al., 2010b) if they were merely sets of regional artifacts. The uniqueness of the PDRP was demonstrated by its correlation with analogous patterns derived in two independent iPD populations ($R^2 \geq 0.80$), whereas there is no analogous correlation between the PDRP and the patterns derived for two atypical parkinsonian syndromes (Eckert et al., 2008): multiple system atrophy (MSA) ($R^2 = 0.008$) and progressive supranuclear palsy (PSP) ($R^2 = 0.097$) (Fig.14).

6. Conclusion

Incorrect application of SSM and other multivariate imaging approaches can lead to misinterpretation of simple and powerful analytical tools. Overall, the robustness of the derived disease-related metabolic networks is remarkable. We have shown that even in modified applications of the SSM method, the PDRP pattern emerges largely unaltered as a misplaced component or as a set of component fragments. In this and previous studies, we have seen that it is an undisputable disease characteristic in independent populations of PD patients (cf. Moeller et al., 1999; Eidelberg, 2009). Although SSM covariance patterns for PD may bear a superficial resemblance to images of networks identified in other neurological conditions, they must be evaluated as whole volumes of voxel weights in 3D space before comparisons are made. More often, visual differences (from normal and between conditions) that are not evident in raw data are apparent in SSM patterns. For instance, the contrast is evident even in the single plane display of the characteristic SSM patterns associated with three independent parkinsonian syndromes, normalized to a common color stripe (Fig. 14). In view of the proven utility of SSM in the differential

diagnosis of the parkinsonian movement disorders and in the objective assessment of disease progression and treatment effects in these and related, the value of this approach cannot be disputed.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Appendix A SVD of the SRP matrix

The $M \times M$ *subject by subject* covariance matrix $\mathbf{S}_{\text{sub}}$ of the **SRP** matrix has elements $S_{ss'}$ determined from the following equation:

$$S_{ss'} = \sum_v (\mathbf{SRP}_{sv} \times \mathbf{SRP}_{s'v}), \text{ where } s \text{ and } s' \text{ are two different subject indices.}$$

We can express this equation in matrix format as

$$\mathbf{S}_{\text{sub}} = \mathbf{SRP} \mathbf{SRP}^T. \quad (\text{A1})$$

An eigenvalue decomposition of the matrix $\mathbf{S}_{\text{sub}}$ is performed to derive eigenvalues (λ_k , $k=1, \dots, M$) and eigenvectors ($\mathbf{e}_k$, $k=1, \dots, M$) (Rencher, 1995; Jolliffe, 2002):

$$\mathbf{S}_{\text{sub}} \mathbf{e}_k = \lambda_k \mathbf{e}_k \quad (\text{A2})$$

The elements of the $\mathbf{e}_k$ vectors correspond to the subject scaling factors SSF_{ks} (i.e., the PC scalars) as defined in (Moeller and Strother, 1991; Alexander and Moeller, 1994). Left multiplying both sides of this equation by $\mathbf{SRP}^T$ we obtain,

$$\begin{aligned} \mathbf{SRP}^T \mathbf{S}_{\text{sub}} \mathbf{e}_k &= \mathbf{SRP}^T \lambda_k \mathbf{e}_k = \lambda_k \mathbf{SRP}^T \mathbf{e}_k \\ (\mathbf{SRP}^T \mathbf{SRP}) \mathbf{SRP}^T \mathbf{e}_k &= \lambda_k \mathbf{SRP}^T \mathbf{e}_k. \end{aligned}$$

Noting that $(\mathbf{SRP}^T \mathbf{SRP})$ is the $(N \times N)$ voxel by voxel covariance related matrix $\mathbf{S}_{\text{vox}}$ [with elements $S_{vv'} = \sum_s (\mathbf{SRP}_{sv} \times \mathbf{SRP}_{s'v'})$], we see that the vectors $\mathbf{SRP}^T \mathbf{e}_k$ (denoted in SSM as Group Invariant Subprofile vectors $\mathbf{GIS}_k$) are eigenvectors of $\mathbf{S}_{\text{vox}}$ with the same eigenvalues λ_k derived above:

$$\mathbf{S}_{\text{vox}} \mathbf{GIS}_k = \lambda_k \mathbf{GIS}_k \quad \text{where, } \mathbf{S}_{\text{vox}} = (\mathbf{SRP}^T \mathbf{SRP}) \text{ and } \mathbf{GIS}_k \equiv \mathbf{SRP}^T \mathbf{e}_k. \quad (\text{A3})$$

The eigenvalues are listed in descending order of significance corresponding to decreasing contributions to the total variance. The variance accounted for each vector (vaf_k) is determined from the relative value of each λ_k to the sum of the eigenvalues:

$$\text{vaf}_k = \lambda_k / (\lambda_1 + \lambda_2 + \dots + \lambda_M). \quad (\text{A4})$$

Each of the unitized $\mathbf{e}_k$ vectors are weighted by the square root of their corresponding λ_k values to yield the subject score vectors (**Score_k**) whose elements represent the subject dependent expression of each pattern vector **GIS_k**.

$$\mathbf{Score}_k = \sqrt{\lambda_k} \mathbf{e}_k \quad (\text{A5})$$

Abbreviations

SSM	Scaled Subprofile Model
PCA	principal components analysis
GMR	global metabolic rate
PDRP	Parkinson's disease-related pattern
GMP	Group Mean Profile
SRP	Subject Residual Profile
TPR	topographic profile rating
AIC	Akaike information criterion
ROI	regions-of-interest
MCI	mild cognitive impairment
MSA	multiple system atrophy
PSP	progressive supranuclear palsy
SVD	singular value decomposition

References

- Abdi, H. Bonferroni and Sidak Corrections for Multiple Comparisons. Sage; Thousand Oaks, CA: 2007.
- Akaike H. A new look at the statistical model identification. IEEE Transactions on Automatic Control. 1974; 19:716–723.
- Alexander GE, Moeller JR. Application of the scaled subprofile model to functional imaging in neuropsychiatric disorders: A principal component approach to modeling brain function in disease. Hum Brain Mapp. 1994; 2:1–16.
- Asanuma K, Ma Y, Huang C, Carbon-Correll M, Edwards C, Raymond D, Bressman SB, Moeller JR, Eidelberg D. The metabolic pathology of dopa-responsive dystonia. Ann Neurol. 2005; 57:596–600. [PubMed: 15786454]
- Asanuma K, Tang C, Ma Y, Dhawan V, Mattis P, Edwards C, Kaplitt MG, Feigin A, Eidelberg D. Network modulation in the treatment of Parkinson's disease. Brain. 2006; 129:2667–2678. [PubMed: 16844713]
- Borghammer P, Chakravarty M, Jonsdottir KY, Sato N, Matsuda H, Ito K, Arahata Y, Kato T, Gjedde A. Cortical hypometabolism and hypoperfusion in Parkinson's disease is extensive: probably even at early disease stages. Brain Struct Funct. 2010; 214:303–317. [PubMed: 20361208]
- Borghammer P, Cumming P, Aanerud J, Gjedde A. Artefactual subcortical hyperperfusion in PET studies normalized to global mean: lessons from Parkinson's disease. Neuroimage. 2009; 45:249–257. [PubMed: 18718541]
- Borghammer P, Jonsdottir KY, Cumming P, Ostergaard K, Vang K, Ashkanian M, Vafaee M, Iversen P, Gjedde A. Normalization in PET group comparison studies--the importance of a valid reference region. Neuroimage. 2008; 40:529–540. [PubMed: 18258457]

- Cadima J, Jolliffe I. On relationships between uncentered and column-centred principal component analysis. *Pakistan Journal of Statistics*. 2009; 25:473–503.
- Cattell RB. The scree test for the number of factors. *Multiv Behav Res*. 1966; 1:245–276.
- Eckert T, Barnes A, Dhawan V, Frucht S, Gordon MF, Feigin AS, Eidelberg D. FDG PET in the differential diagnosis of parkinsonian disorders. *Neuroimage*. 2005; 26:912–921. [PubMed: 15955501]
- Eckert T, Tang C, Ma Y, Brown N, Lin T, Frucht S, Feigin A, Eidelberg D. Abnormal metabolic networks in atypical parkinsonism. *Mov Disord*. 2008; 23:727–733. [PubMed: 18186116]
- Eckert T, Van Laere K, Tang C, Lewis DE, Edwards C, Santens P, Eidelberg D. Quantification of Parkinson's disease-related network expression with ECD SPECT. *Eur J Nucl Med Mol Imaging*. 2007; 34:496–501. [PubMed: 17096095]
- Eidelberg D. Metabolic brain networks in neurodegenerative disorders: a functional imaging approach. *Trends Neurosci*. 2009; 32:548–557. [PubMed: 19765835]
- Eidelberg D, Moeller JR, Dhawan V, Spetsieris P, Takikawa S, Ishikawa T, Chaly T, Robeson W, Margouleff D, Przedborski S, et al. The metabolic topography of parkinsonism. *J Cereb Blood Flow Metab*. 1994; 14:783–801. [PubMed: 8063874]
- Eidelberg D, Moeller JR, Ishikawa T, Dhawan V, Spetsieris P, Chaly T, Robeson W, Dahl JR, Margouleff D. Assessment of disease severity in parkinsonism with fluorine-18-fluorodeoxyglucose and PET. *J Nucl Med*. 1995; 36:378–383. [PubMed: 7884498]
- Feigin A, Antonini A, Fukuda M, De Notaris R, Benti R, Pezzoli G, Mentis MJ, Moeller JR, Eidelberg D. Tc-99m ethylene cysteinate dimer SPECT in the differential diagnosis of parkinsonism. *Mov Disord*. 2002; 17:1265–1270. [PubMed: 12465066]
- Feigin A, Kaplitt MG, Tang C, Lin T, Mattis P, Dhawan V, Doring MJ, Eidelberg D. Modulation of metabolic brain networks after subthalamic gene therapy for Parkinson's disease. *Proc Natl Acad Sci U S A*. 2007a; 104:19559–19564. [PubMed: 18042721]
- Feigin A, Tang C, Ma Y, Mattis P, Zgaljardic D, Guttman M, Paulsen JS, Dhawan V, Eidelberg D. Thalamic metabolism and symptom onset in preclinical Huntington's disease. *Brain*. 2007b; 130:2858–2867. [PubMed: 17893097]
- Friston KJ, Holmes AP, Worsley KJ, Poline J-P, Frith CD, Frackowiak RSJ. Statistical parametric maps in functional imaging: a general linear approach. *Hum Brain Mapp*. 1995; 2:189–210.
- Gwadry, F.; Berenstein, C.; Horn, JV.; Braun, A. Implementation and Application of Principal Component Analysis on Functional Neuroimaging Data: Institute for Systems Research Technical Reports. 2001. p. 1-27.
- Habeck C, Foster NL, Perneczky R, Kurz A, Alexopoulos P, Koeppe RA, Drzezga A, Stern Y. Multivariate and univariate neuroimaging biomarkers of Alzheimer's disease. *Neuroimage*. 2008; 40:1503–1515. [PubMed: 18343688]
- Hastie, T.; Tibshirani, R.; Friedman, J. The Elements of Statistical Learning: Data Mining, Inference, and Prediction. Springer; New York: 2001.
- Hirano S, Asanuma K, Ma Y, Tang C, Feigin A, Dhawan V, Carbon M, Eidelberg D. Dissociation of metabolic and neurovascular responses to levodopa in the treatment of Parkinson's disease. *J Neurosci*. 2008; 28:4201–4209. [PubMed: 18417699]
- Huang C, Dyke JP, Pan H, Feigin A, Pourfar M, Eidelberg D. Assessing network activity in Parkinson's disease with arterial spin labeling MRI. *Neuroimage*. 2007a; 36:S111.
- Huang C, Mattis P, Perrine K, Brown N, Dhawan V, Eidelberg D. Metabolic abnormalities associated with mild cognitive impairment in Parkinson disease. *Neurology*. 2008; 70:1470–1477. [PubMed: 18367705]
- Huang C, Mattis P, Tang C, Perrine K, Carbon M, Eidelberg D. Metabolic brain networks associated with cognitive function in Parkinson's disease. *Neuroimage*. 2007b; 34:714–723. [PubMed: 17113310]
- Huang C, Tang C, Feigin A, Lesser M, Ma Y, Pourfar M, Dhawan V, Eidelberg D. Changes in network activity with the progression of Parkinson's disease. *Brain*. 2007c; 130:1834–1846. [PubMed: 17470495]
- Jolliffe, IT. Principal Components Analysis, 2nd edition, Springer Series in Statistics. Springer-Verlag; New York: 2002.

- Kazmierczak JB. Analyse logarithmique deux exemples d'application. *Rev Statistique Appliquee*. 1985; 33:13–24.
- Lin TP, Carbon M, Tang C, Mogilner AY, Sterio D, Beric A, Dhawan V, Eidelberg D. Metabolic correlates of subthalamic nucleus activity in Parkinson's disease. *Brain*. 2008; 131:1373–1380. [PubMed: 18400841]
- Ma Y, Tang C, Moeller JR, Eidelberg D. Abnormal regional brain function in Parkinson's disease: truth or fiction? *Neuroimage*. 2009; 45:260–266. [PubMed: 18992824]
- Ma Y, Tang C, Spetsieris PG, Dhawan V, Eidelberg D. Abnormal metabolic network activity in Parkinson's disease: test-retest reproducibility. *J Cereb Blood Flow Metab*. 2007; 27:597–605. [PubMed: 16804550]
- Miranda AA, Le Borgne Y-A, Bontempi G. New routes from minimal approximation error to principal components. *Neural Processing Letters*. 2008; 27:197–207.
- Moeller JR, Eidelberg D. Divergent expression of regional metabolic topographies in Parkinson's disease and normal ageing. *Brain*. 1997; 120(Pt 12):2197–2206. [PubMed: 9448575]
- Moeller JR, Nakamura T, Mentis MJ, Dhawan V, Spetsieris P, Antonini A, Missimer J, Leenders KL, Eidelberg D. Reproducibility of regional metabolic covariance patterns: comparison of four populations. *J Nucl Med*. 1999; 40:1264–1269. [PubMed: 10450676]
- Moeller JR, Strother SC. A regional covariance approach to the analysis of functional patterns in positron emission tomographic data. *J Cereb Blood Flow Metab*. 1991; 11:A121–135. [PubMed: 1997480]
- Mosconi L, Tsui WH, Herholz K, Pupi A, Drzezga A, Lucignani G, Reiman EM, Holthoff V, Kalbe E, Sorbi S, Diehl-Schmid J, Perneczky R, Clerici F, Caselli R, Beuthien-Baumann B, Kurz A, Minoshima S, de Leon MJ. Multicenter standardized 18F-FDG PET diagnosis of mild cognitive impairment, Alzheimer's disease, and other dementias. *J Nucl Med*. 2008; 49:390–398. [PubMed: 18287270]
- Petersson KM, Nichols TE, Poline JB, Holmes AP. Statistical limitations in functional neuroimaging. I. Non-inferential methods and statistical models. *Philos Trans R Soc Lond B Biol Sci*. 1999; 354:1239–1260. [PubMed: 10466149]
- Poston KL, Eidelberg D. Network biomarkers for the diagnosis and treatment of movement disorders. *Neurobiol Dis*. 2009; 35:141–147. [PubMed: 19013242]
- Rencher, AC. *Methods of Multivariate Analysis*. 2nd edition. Wiley-Interscience; New York: 1995.
- Spetsieris, P.; Dhawan, V.; Eidelberg, D. Three-fold cross-validation of parkinsonian brain patterns.. *Proc 32nd Ann Int Conf of the IEEE EMBS*; 2010. p. 2906-2909.
- Spetsieris P, Ma Y, Dhawan V, Moeller JR, Eidelberg D. Highly automated computer-aided diagnosis of neurological disorders using functional brain imaging. *Proc SPIE: Medical Imaging*. 2006; 6144:61445M61441–61412.
- Spetsieris PG, Ma Y, Dhawan V, Eidelberg D. Differential diagnosis of parkinsonian syndromes using PCA-based functional imaging features. *Neuroimage*. 2009; 45:1241–1252. [PubMed: 19349238]
- Tang CC, Poston KL, Dhawan V, Eidelberg D. Abnormalities in metabolic network activity precede the onset of motor symptoms in Parkinson's disease. *J Neurosci*. 2010a; 30:1049–1056. [PubMed: 20089913]
- Tang CC, Poston KL, Eckert T, Feigin A, Frucht S, Gudesblatt M, Dhawan V, Lesser M, Vonsattel JP, Fahn S, Eidelberg D. Differential diagnosis of parkinsonism: a metabolic imaging study using pattern analysis. *Lancet Neurol*. 2010b; 9:149–158. [PubMed: 20061183]
- Tolosa E, Wenning G, Poewe W. The diagnosis of Parkinson's disease. *Lancet Neurol*. 2006; 5:75–86. [PubMed: 16361025]
- Trost M, Su S, Su P, Yen RF, Tseng HM, Barnes A, Ma Y, Eidelberg D. Network modulation by the subthalamic nucleus in the treatment of Parkinson's disease. *Neuroimage*. 2006; 31:301–307. [PubMed: 16466936]

Reference PDRP **Group 1, 33 Normals, 33 iPD, Mask T=.35**

Fig. 1. Parkinson's Disease-Related Pattern (PDRP). Axial, coronal and sagittal views through the origin of MNI space of the first principal component (GIS1, vaf 21.4%) of the Group 1 SSM analysis. The associated patient/normal scores t -test p -value was $p=1.94e^{-12}$. The covariance pattern indicates relatively higher component deviations in the cerebellum, pons, thalamus and basal ganglia and lower values in the frontal, premotor, parietal and occipital regions.

Fig. 2.

Depiction of axial views (z=-26mm and z=-2mm of MNI space) of principal component 1 derived in Group 1 SSM analysis using masks corresponding to a variable threshold parameter. Corresponding p-values of associated subject score *t*-tests indicated optimum discrimination of patients versus normal controls for T=.35 ($p=1.94e^{-12}$).

Fig. 3.

SSM analysis Group 1 – PD33. Depiction of four axial slices of two PCs corresponding to a major and minor source of variance (vaf) and their combination from the SSM analysis of Group 1. Associated scores of the first PC significantly discriminated patient from normal controls (PDRP-GIS 1, vaf=21.4%, $p=1.9e^{-12}$). Higher discrimination is achieved (GIS1,8, $p=2.4e^{-16}$) by *logistic* combination with a smaller component (GIS 8, vaf=2.6%, $p=0.004$).

Fig. 4.
PD33 – Non-Log-Non-Scaled PCA Group 1. Without log transformation or previous global pre-scaling, the PCA analysis of Group 1 results in numerous components with lesser discriminative capacity but higher when combined. The vaf values for PCs 1 to 6 and some combined PCs are noted followed by 3 values indicating the p-values of associated Group 1 scores (p(33)) and Group 2 TPR scores (p(30)) and the R^2 voxel correlation with the reference PDRP pattern (see also suppl. Table 2).

Fig. 5.

Log PCA and Non-Log-Pre-Scaled PCA Group 1. Comparison of (a) standard SSM (log PCA) and modified SSM with non-log data and (b) global GMR pre-scaling and (c) CMR pre-scaling. Analysis yielded two PCs with p values < 0.01 in each case.

Fig. 6.

Log PCA and Non-Log-Pre-Scaled PCA Group 2.

Comparison of (a) standard SSM (log PCA) and modified SSM with non-log data and (b) global GMR pre-scaling and (c) CMR pre-scaling. Analysis yielded two or three significant PCs with p values <0.07 that yielded a similar pattern when combined ($p \sim e^{-7}$).

Modified SSM , 33 Normals, 33 iPD, Subject Uncentered Log Data

Fig. 7.

Modified SSM, Subject uncentered log data Group 1. Failure to subtract the subject log mean results in the concentration of most of the variance (vaf=97%) in the first PC that does not discriminate patients from normals (p=0.45) and a shift of the PDRP factor to PC 2 corresponding to a small fraction of the variance (vaf=0.64%) but with essentially the same associated score discrimination as the PDRP (p= 1.912 e⁻¹²).

a

Bivariate Fit of GIS 1 Scores by LGMR

b

Bivariate Fit of GIS 2 Scores by LGMR

Fig. 8. Correlation of Group 1 LGMR by a) GIS1 scores and b) GIS 2 scores for uncentered log data. Near perfect correlation of the log data GMR with GIS 1 Scores ($R^2 \approx 1.0$, $p < 0.0001$) (Fig. 8a) and no correlation with GIS 2 scores ($R^2 < 0.0001$, $p = 0.999$) (Fig. 8b).

Fig. 9. Modified SSM using Group 1 Cerebellar mean decentered data. Subtraction of the mean of the log of the subject cerebellar region values resulted in a fragmenting of PCs into PC1 (vaf = 46.6%, $p = 6.6e^{-4}$) and PC2 (vaf = 10%, $p = 2.8e^{-8}$). The combined PCs (GIS1-2) correlated significantly with the PDRP ($R^2 = 0.99$).

Global Metabolic Rate in NL, MCI & PDD

Fig. 10a. Mean GMR in normal subjects (NL), PD patients with normal cognitive function (MCI(-)), single domain mild cognitive impairment (MCI(s)), with mild cognitive impairment (MCI(m)) and with signs of dementia (PDD) (see Huang et al., 2008; Eidelberg, 2009 for details)

Fig. 10b.
GMR values and PDRP/PDCP scores plotted against disease duration (see Eidelberg, 2009 for details).

Fig. 11.

Comparison of PD-related metabolic patterns identified in different derivation groups (normalized display superimposed on MRI background). The p-values result from two sample t-tests between normal and PD patient scores in the derivation groups. R^2 is the Pearson's squared correlation coefficient of corresponding voxels between each pattern and the PDRP.

Fig. 12.

Red, Blue Component and Whole PDD_RP mean scores for NL vs. PD in Group 3 and prospective TPR values in Groups 1 and 2 and GMR mean values. Asterisks represent the p-value of a two-sample *t*-test between normals and PD: ****<0.0001, ***<0.001, * > 0.1.

Fig. 13.

PDD Red-Area Pattern displayed on an MRI background. a) PDD-RP Red Component. This pattern represents only positive weighted voxels of the whole brain PDD-RP, b) Mask corresponding to the PDD-RP Red Pattern voxels, c) Rederived pattern from SSM analysis limited to masked area in Group 3.

Fig. 14.

Parkinsonian GIS network biomarkers for iPD, MSA, PSP displayed on an MRI background. The uniqueness of SSM derived disease patterns is visually demonstrated in these corresponding axial views ($Z=0$) of representative patterns derived for three distinct parkinsonian syndromes normalized to a common color scale. Participants belonged to three separate disease groups, PD Group 1 (33 PD, 33 NL), MSA (14 MSA, 14 NL) and PSP (10 PSP, 10 NL).

Table 1

Group demographics

Group		Gender Male/Female	Age Years (stdev)	Disease Duration Years (stdev)
Group 1				
PD	[33]	22/11	57.2 (8.2)	9.2 (3.6)
NL	[33]	10/23	55.0 (13.4)	
Group 2				
PD	[30]	19/11	56.8 (10.4)	2.2 (1.1)
NL	[30]	14/16	53.7 (13.2)	
Group 3				
PD	[9]	5/4	64.4 (12.1)	14.6 (5.8)
NL	[9]	5/4	65.7 (9.4)	

Table 2

Group 1: PCA - with pre-scaling and no log transformation

	GMR PRE-SCALING ^b		CER PRE-SCALING ^c		STANDARD ^a	
	gPC ₁	gPC ₈	cPC ₁	cPC ₈	PDRP	PC ₈
Val%	22	2.7	22.7	2.7	21.4	2.6
90 p (33)	1.95e ⁻¹²	0.01	5.1e ⁻¹³	0.004	1.94e ⁻¹²	0.004
R ² (PDRP)						
VOX	0.984	0.000	0.969	0.000	1	0.000
SCORES	0.998	0.000	0.981	0.000	1	0.000
TPR p (30)	2e ⁻⁴	.92	7e ⁻⁴	.98	4.8e ⁻⁷	.668
TPR p (9)	1.8e ⁻³	.23	7e ⁻⁴	.45	1e ⁻⁴	.387

^alog transformed data

^bglobal ratio normalization

^ccerebellar ratio normalization

Table 3Group 1: R^2 [vox, score] correlations

	PC_{1-3}^0	gPC_1^b	cPC_1^c	$PDRP^a$
PC_{1-3}^0	1, 1	.73, .61	.70, .64	.73, .60
gPC_1^b	.73, .61	1, 1	.98, .98	.98, .998
cPC_1^c	.70, .64	.98, .98	1, 1	.97, .98
$PDRP^a$	.73, .60	.98, .998	.97, .98	1, 1

 0
no transformation a log transformed data b GMR pre-scaled c CER pre-scaled

Table 4

Group 3: SSM analysis, log data

	WHOLE PC ₁₂ (Gp 3) Vaf (49.9%)	RED PC ₁₂ (Gp 3)	BLUE PC ₁₂ (Gp 3)	PDRP (Gp 1)	PC ₁₂ (Gp 2)
<i>p</i> (9)	2e ⁻⁴	1e ⁻⁴	3e ⁻⁴	1.1e ⁻⁴⁺	3e ⁻⁴⁺
<i>p</i> (33) ⁺	1.5e ⁻¹²⁺	2.3e ⁻¹³⁺	6.6e ⁻¹¹⁺	1.9 e ⁻¹²⁺⁺	1.04 e ⁻¹²⁺
<i>p</i> (30) ⁺	5.1e ⁻⁷⁺	1.2e ⁻⁷⁺	3.5e ⁻⁶⁺	4.8 e ⁻⁷⁺	3.09 e ⁻⁷⁺⁺
<i>R</i> ² (PDRP)					
VOX	.82	.55	.60	1	.80
SCORES ⁺	.98	.98	.98	1 ⁺	.98 ⁺

⁺ TPR score values

⁺⁺ direct (non-TPR)