Abnormal network topographies and changes in global activity: Absence of a causal relationship

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Changes in regional brain activity can be observed following global normalization procedures to reduce variability in the data. In particular, spurious regional differences may appear when scans from patients with low global activity are compared to those from healthy subjects. It has thus been suggested that the consistent increases in subcortical activity that characterize the abnormal Parkinson’s disease-related metabolic covariance pattern (PDRP) are artifacts of global normalization, and that similar topographies can be identified in scans from healthy subjects with varying global activity. To address this issue, we examined the effects of experimental reductions in global metabolic activity on PDRP expression. Ten healthy subjects underwent 18F-fluorodeoxyglucose PET in wakefulness and following sleep induction. In all subjects, the global metabolic rate (GMR) declined with sleep (mean - 34%, range: -17 to -56%), exceeding the test–retest differences of the measure (p<0.001). By contrast, sleep–wake differences in PDRP expression did not differ from test–retest differences, and did not correlate (R^2 = 0.04) with concurrent declines in global metabolic activity. Indeed, despite significant GMR reductions in sleep, PDRP values remained within the normal range. Likewise, voxel weights on the principal component patterns resulting from combined analysis of the sleep and wake scans did not correlate (R^2 = 0.07) with the corresponding regional loadings on the PDRP topography. In aggregate, the data demonstrate that abnormal PDRP expression is not induced by reductions in global activity. Moreover, significant declines in GMR are not associated with the appearance of PDRP-like spatial topographies.

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Introduction

Spatial covariance analysis of metabolic images has provided valuable insights into the network-level abnormalities that underlie different brain disorders (Eidelberg, 2009). In recent years, a rigorous computational framework has been developed to identify specific disease-related metabolic topographies, which can be used to track disease progression and monitor treatment responses (Habeck and Stern, 2010; Spetsieris and Eidelberg, 2011). Of the neurodegenerative disorders, Parkinson’s disease (PD) has been the most extensively studied from a network standpoint (Niethammer and Eidelberg, in press; Poston and Eidelberg, 2012). This disorder is associated with an abnormal metabolic network (Fig. 1A) characterized by increased activity in the putamen, globus pallidus, ventral thalamus, dorsal pons and cerebellum, with relative reductions in premotor and parietal association regions. This distinctive and highly replicable topography, known as the PD-related covariance pattern (PDRP), is thought to represent disease-specific abnormalities of local synaptic activity analogous to those described in experimental animal models of the disorder (e.g., Guigoni et al., 2005). Indeed, a topographically homologous metabolic network has recently been reported in non-human primates with experimental Parkinsonism (Ma et al., 2007). The intimate relationship between the PDRP and the underlying disease process has been substantiated by the close correlation that has been observed between network activity and intraoperative cell recordings in PD patients (Lin et al., 2008). Further support for a biological interpretation of the PDRP topography is provided by the stereotyped dialtual changes that have been noted following surgical antiparkinsonian interventions targeting discrete elements of this network (Asanuma et al., 2006; Eidelberg et al., 1996; Fukuda et al., 2001; Mure et al., 2011; Su et al., 2001; Trost et al., 2006).

The PDRP topography has also been thought to be an artifact of global normalization (Borghammer et al., 2008, 2009a, 2009b), a commonly utilized means of reducing intersubject variability in the analysis of brain imaging data. It is well appreciated that this preprocessing step, particularly ratio normalization by the global mean value for each scan, can potentially elevate measurements of local activity in healthy brain regions (e.g., Petersson et al., 1999), and also affect the magnitude and direction of resting state correlations (Fox et al., 2009; Murphy et al., 2009). This concern is particularly relevant in studies of progressive neurodegenerative disorders, in which...
PET images from 33 PD patients and 33 age-matched healthy volunteer subjects (Ma et al., associated with relative reductions (the putamen, globus pallidus (GP), thalamus, pons, cerebellum and sensorimotor cortex) of the voxel weights on the pattern was thresholded at Z=3.61, p<0.001 and overlaid on T1-weighted MR-template images]. B. Global metabolic rate (GMR) for parieto-occipital association regions. [In this combined group analysis, the PDRP was comprised of healthy control (CN1) subjects scanned in wakefulness. Analogous values are displayed for PD patients scanned as part of a test (gray bar)/retest (black bar) study (Ma et al., 2007), and for a separate reference group (white bar) of waking PD controls (CN2). A significant decline in GMR (p<0.001) was observed during sleep induction, whereas no change was evident between test and retest (p=0.71). C. The corresponding sleep–wake changes in PDRP expression did not differ from the test–retest differences of this measure (p=0.26).

Methods

Subjects

Ten healthy volunteer subjects (age: 54.4±16.1 years (mean±SD), range: 31.6–72.6 years) were scanned with 18F-fluorodeoxyglucose (FDG) PET in sleep and wakefulness (see below). Scans from the additional groups of subjects were used for reference: (1) a healthy control group comprised of 10 normal subjects (CN1; age: 53.2±11.3 years, range: 32.8–70.8 years); (2) a PD patient control group comprised of 10 PD subjects (CN2; age: 56.8±7.0 years, range: 38.5–65.8 years); and (3) a test–retest group of 10 PD subjects (age: 64.1±8.9 years, range: 50.9–77.4 years) who underwent repeat metabolic imaging twice within three weeks as part of a previously published test–retest study (Ma et al., 2007). The reference studies were all conducted in wakefulness.

Ethical permission to conduct these studies was obtained from the Institutional Review Board of the North Shore-Long Island Jewish Health System. Written informed consent was obtained from each participant following a detailed explanation of the procedures.

Positron emission tomography

Imaging in the waking state was performed in three-dimensional (3D) mode using the GE Advance tomograph (GE Medical Systems, Milwaukee, WI) at North Shore University Hospital, Manhasset, NY as detailed elsewhere (e.g., Lin et al., 2008; Ma et al., 2007). All subjects fasted overnight before PET imaging; in the PD patients, antiparkinsonian medications were withheld for at least 12 h before the start of imaging. The studies were performed in a dimly lit room and with minimal auditory stimulation. Global and regional rates of cerebral glucose utilization were computed for each scan on a voxel-by-voxel basis. Imaging during sleep was performed as described previously (Hutchinson et al., 2000). In the subjects undergoing both sleep and wake studies, scanning took place on consecutive days in random order. For sleep induction, subjects received 100 mg–200 mg of secobarbital orally approximately 1 h before imaging. The sleep state during imaging was monitored with EEG recordings; radiotracer was injected only after the subjects were judged to be in stage II sleep.

Data analysis

Metabolic images were standardized to the Montreal Neurological Institute template using an isotropic spatial smoothing filter (10 mm
full width at half maximum); sleep and wake scans were aligned automatically. All image processing was performed using Statistical Parametric Mapping (SPM) software (Wellcome Department of Cognitive Neurology, London, UK) running in MATLAB (Mathworks, Natick, MA).

We first determined whether abnormal elevations in PDRP expression could be induced experimentally in healthy subjects by lowering global activity. To this end, we quantified PDRP expression in each subject/condition using an automated voxel-based algorithm (software available at http://www.fil.ion.ucl.ac.uk/spm/ext/#SSM) as described previously (Eidelberg, 2009; Ma et al., 2007). These computations were performed blind to subject, group (PD/normal), and experimental condition (sleep/wake; test/retest). The resulting PDRP values were standardized by z-transformation with respect to the 33 healthy volunteer subjects used in deriving the pattern (Ma et al., 2007).

For each scan we computed GMR, which can be influenced by individual variation in injected activity, body weight, and the state of alertness of the subject during radiotracer uptake. Because it is possible for the measured GMR values to be influenced by large-scale topographic effects in the data, the SSM provides an additional global measure that is region independent, i.e., that scales all voxels equally in a given scan (Alexander and Moeller, 1994; Moeller and Strother, 1991; Moeller et al., 1996). That is, the SSM analysis partitions GMR variance into global and regional effects. The global variance is represented by the global scaling factor (GSF) and the topographic profiles, i.e., the principal component (PC) patterns in the data. Operationally, the GSF is obtained by saving the unstandardized residuals of a multiple regression analysis where the PC scalars (i.e., the subject scores) are used to predict the GMR for each subject/condition. Hence in the current study, the GSF represented that aspect of GMR variance that was not explained by individual differences in the expression of the first several PCs (together accounting for the upper 50% of the subject × voxel variance in the combined sleep–wake data (see below)). The significance of sleep–wake changes in PDRP expression and in global activity was respectively determined by comparing the between-condition differences to the test–retest differences of each measure using 2 × 2 repeated measures analysis of variance (RMANOVA) with post-hoc Bonferroni tests. This approach was also used to directly compute sleep–wake differences in PDRP expression with the concurrent changes in global activity. The relationship between changes in these variables was additionally evaluated by correlation analysis using Pearson product–moment correlation coefficients. These analyses were implemented in SPSS 17.0 for Windows (SPSS, Chicago, IL) and were considered significant for p < 0.05.

In a separate set of analyses, we determined whether PDRP-like topographies are expressed in healthy subjects with low global metabolic activity. To this end, we combined the sleep and wake scans into a single group and conducted a voxel-wise SSM analysis. In this analysis, the relationship between mean spatial image, global scaling factor and the PCA component is defined by the following equation:

\[ P_{ij} = GMR_i + GMP_j + SRP_{ij} \]

where \( i \) is the subject index and \( j \) is the voxel index. The subject log data (\( P_j \)) can be expressed as a sum of subject and spatial mean values and a subject residual profile term (\( SRP_{ij} \)) that is submitted to PCA. GMR, is the subject whole brain mean log value. The subject GMR value is subtracted from the corresponding subject voxel values and the GMP \( j \) is then evaluated as the spatial group mean value of voxel \( j \). Details of the SSM analysis have been reported elsewhere (Eidelberg, 2009; Spetsieris and Eidelberg, 2011).

Logistical regression analysis was then performed on subject scores for the patterns that account for the largest effects in the data (typically the first four or five PCs, together accounting for approximately 50% of the subject × voxel variance) to identify that which maximally discriminated between the wake and sleep conditions. Specifically, subject scores for each PC were entered singly and in all possible combinations as the independent variables into a series of logistical regression models with the experimental condition (wake/sleep) as the dependent variable. The model with the lowest Akaike Information Criterion (Burnham and Anderson, 2002) value was selected and significance was determined by the p-value of the likelihood ratio test.

Voxel weights on the PCs contributing to the selected model were linearly combined according to the regression coefficients on the respective subject scores (Moeller et al., 1996). Voxel weights on this topography were tested for reliability using bootstrap resampling with 1000 iterations (Efron and Tibshirani, 1994; Habeck and Stern, 2010). Forward computations of pattern expression in prospective scan data from individual subjects were performed as described above. All subject scores for the resulting sleep/wake discrimination pattern were z-transformed with respect to the waking scans of the derivation cohort such that the mean value was zero with a standard deviation of one.

Following spatial covariance analysis, we interrogated the space spanned by the major pattern vectors for the presence of topographies with “PDRP-like” regional features. This was accomplished by correlating the loadings on each of these patterns with the corresponding PDRP weights using a voxel-wise routine described elsewhere (Mure et al., 2011). The \( R^2 \) value for each correlation was computed across all voxels with non-zero region weights. For this calculation, images are assumed to be previously spatially normalized in 3D space and only voxels that are not zero in both images are considered. Voxel weights on both covariance patterns are represented as data vectors of equal size, involving approximately 150,000 voxels for the masked non-zero gray matter space common to the two patterns. Voxels from each image are formatted into a single vector by appending successive rows in each plane of the image. Voxel weights on each pattern, i.e., the regional elements of the respective data vectors, were z-scored as per our routine (Spetsieris and Eidelberg, 2011). The two vectors are input into the MATLAB statistical routine ‘corr’ to calculate the Pearson’s product–moment correlation coefficient. The same procedure was separately applied to the sleep/wake discrimination pattern to determine whether its regional loadings correlated with those on the PDRP.

**Results**

Sleep induction (Fig. 1B) produced a decline in GMR of 33.9 ± 13.9% (mean ± SD, range: –16.9 to –55.8%) relative to the baseline waking condition (p = 0.001). These changes were significantly greater than the test–retest differences of this measure (F(1,18) = 22.5, p < 0.001; 2 × 2 RMANOVA). Waking GMR in this group did not differ from the corresponding values measured in the healthy and PD control cohorts (p = 0.38). By contrast, PDRP expression measured concurrently in these subjects (Fig. 1C) did not change significantly during sleep induction (p = 0.11). The changes in network activity observed during sleep induction did not differ from the test–retest differences of this measure (F(1,18) = 1.4, p = 0.26; 2 × 2 RMANOVA). Indeed, the sleep–wake differences in PDRP expression observed in these subjects were substantially smaller in magnitude than the concurrent declines in GMR recorded from the same individuals (F(1,18) = 19.6, p < 0.001; 2 × 2 RMANOVA). Similarly, the differences in network expression seen during sleep did not correlate with declining global values (\( R^2 = 0.04, p = 0.59 \)). Of note, PDRP expression in these healthy subjects, whether measured in sleep or wakefulness, did not differ from reference values for waking normals in the healthy control group (p = 0.22). On the other hand, PDRP values measured in either condition were lower than reference values for waking patients in the PD control group (p = 0.001).

Spatial covariance analysis of the combined sleep and wake scan data revealed four linearly independent principal components (PCs...
1–4) that together accounted for 58.9% of the subject \( \times \) voxel variance. Comparison of these covariance topographies with the PDRP disclosed no spatial homology between these PCs and the PDRP network (\( R^2 < 0.07 \), voxel-wise correlation analysis). Further analysis revealed the presence of a specific spatial covariance pattern for which subject expression differed across experimental conditions. This topography (Fig. 2A), representing a linear combination of PCs 1–4 (23% variance accounted for), was characterized by covarying reductions in metabolic activity in the cerebellum, medial thalamus, occipital cortex, posterior cingulate gyrus, and in parietal association regions. Voxel weights for the pattern were demonstrated to be reliable by bootstrap resampling (\( p < 0.001 \); 1000 iterations). These values did not correlate (\( R^2 = 0.01 \)) with the corresponding regional loadings on the PDRP network.

In all subjects, the expression of this pattern (Fig. 2B) increased in sleep relative to the baseline waking condition (\( p = 0.002 \), binomial test). Of note, pattern expression during wakefulness was found to be similar (Fig. 2C) to the corresponding values computed in the healthy and PD control cohorts (\( p > 0.41 \)) and in the test and retest PD groups (\( p > 0.43 \)). The increases in pattern expression observed during sleep did not correlate with concurrent changes in PDRP expression (\( R^2 = 0.06, p = 0.50 \)) or global activity (GMR: \( R^2 = 0.02, p = 0.73 \); GSF: \( R^2 = 0.06, p = 0.48 \)).

**Discussion**

The data reported in this study demonstrate that experimentally induced reductions in global metabolic activity are not associated with increased PDRP expression. We found that the changes in PDRP expression that took place during sleep induction were significantly smaller than the concurrent declines in global metabolism that were observed, and did not differ from the test–retest differences of the measure. Indeed, despite the consistent global reductions that occurred with sleep, PDRP expression remained within one SD of the normal mean reference value. In this vein, no correlation was evident between the reductions in global activity that were induced by sleep and concurrent changes in PDRP expression. Lastly, contrary to the proposed hypothesis, declining global activity in healthy individuals was not associated with the appearance of artifactual spatial covariance patterns resembling the disease-related topography.

While PDRP-like topographies were not detected, spatial covariance analysis did reveal a consistent sleep-related metabolic topography in the combined sleep–wake data. This covariance pattern was characterized by reduced metabolic activity in the medial thalamus,
and in posterior cingulate, inferior parietal, and occipital cortex. These regions accord well with the changes in regional cerebral blood flow and glucose utilization reported previously in healthy subjects scanned during non-REM sleep (Braun et al., 1997; Nozinger et al., 2002). While not previously described in relation to physiological sleep, prominent cerebellar metabolic reductions were also noted as part of the currently observed covariance topography. The changes observed in this region as part of the network are likely attributable to the barbiturate sedative that was used for sleep induction (Hutchinson et al., 2000). In any event, the expression of this brain network during sleep proved to be independent of changes in both GMR and GSf, a model-based estimator of the region-free global effect. In fact, sleep–wake changes in these measures of global activity were closely inter-related ($R^2 = 0.96$). Thus, the observed global changes are unlikely to have been driven by topographic (i.e., region-specific) effects associated with the transition to non-REM sleep, the administration of a barbiturate sedative, or both. Moreover, the independence of the observed global and topographic effects suggests that the conclusions of the study can be generalized beyond the context of the sleep induction paradigm that was employed.

On a final note, we draw attention to recent reports confirming the presence of basal ganglia hypermetabolism in early stage PD patients scanned on a high resolution PET instrument (Borghammer et al., 2012; Eggers et al., 2009). These findings, resulting from mass-univariate analyses of the data incorporating different normalization schemes, lend further credence to the validity of the PDRP as a quantitative network biomarker of the disease process. Future studies employing spatial covariance analysis of data acquired on such platforms will help specify the precise anatomy of this abnormal topography.

Conclusion

The data indicate that reductions in global activity, as observed with sleep induction, do not lead to concomitant changes in PDRP expression. Moreover, network analysis of scan data from healthy subjects studied in both sleep and wakefulness did not disclose the presence of artifactual "PDRP-like" spatial topographies. Thus, the network-related increases in subcortical activity that characterize this disease-related covariance pattern cannot be ascribed to group differences in global metabolism.

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