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Metabolic Brain Networks in Neurodegenerative Disorders: A Functional Imaging Approach

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Abstract

Network analysis of functional brain imaging data is an innovative approach to study circuit abnormalities in neurodegenerative diseases. In Parkinson's disease, spatial covariance analysis of resting state metabolic images has identified specific regional patterns associated with motor and cognitive symptoms. With functional imaging, these metabolic networks have recently been used to measure system-related progression and evaluate novel treatment strategies. Network analysis is also being used to characterize specific functional biomarkers for Huntington's disease and Alzheimer's disease. These networks have been particularly helpful in uncovering compensatory mechanisms in genetically at-risk individuals. Ongoing developments in network applications are likely to enhance the role of functional imaging in the investigation of neurodegenerative disorders.

Keywords

brain networks; functional imaging; neurodegenerative diseases; positron emission tomography

Introduction

Functional imaging techniques to localize and quantify abnormalities in brain energy metabolism have been used for many years to study the pathophysiology of neurodegenerative disorders. However, initial enthusiasm for these methods was tempered by analytical challenges stemming from the typically large size of the datasets acquired in imaging experiments, and by the substantial variability in brain activity that exists between subjects and regions [1,2]. An innovative approach to these issues was developed by Moeller and colleagues [3,4] in which these sources of variability were modeled as spatially distributed networks. This method, termed the scaled subprofile model (SSM), is a spatial covariance method based on principal components analysis (PCA) to assess subject-by-region effects in functional brain images. Although this data-driven approach was initially developed for resting state metabolic

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data from prespecified anatomical regions, it has been successfully applied in voxelwise analyses of functional images acquired in activated as well as resting conditions [5-7]. A detailed review of the mathematical principles underlying this approach and its relationship to typical univariate methods like statistical parametric mapping (SPM) has appeared recently [8].

In general terms, the ability to assess functional interactions between brain regions complements the information provided by the univariate methods in comparing scans of patients and controls [9]. SSM/PCA and related multivariate techniques are particularly well suited to the study of circuit disorders, i.e. neurological diseases associated with stereotyped disturbances of brain network organization [10]. Resting state measures of regional glucose utilization provide an index of local synaptic activity as well as the biochemical maintenance processes that dominate this condition [11,12]. The effects of pathology on these functions have greater influence on regional cerebral metabolism and blood flow than physiological and/or hemodynamic factors [13]. It was recognized early on that neurodegenerative processes, even if highly localized, are associated with disease-specific alterations in functional connectivity across the whole brain [4,14]. Given the topographically stereotyped nature of these abnormalities, network approaches have been increasingly used in analyzing metabolic imaging data from these brain disorders. Indeed, with the relatively recent development of voxel-based computational algorithms for unbiased and objective network quantification, these approaches are having a growing impact on imaging neuroscience.

In this review, we discuss recent contributions of spatial covariance analysis of metabolic imaging data to the study of the systems-level changes in brain function that characterize neurodegenerative disorders. This approach has provided major insights into the circuit abnormalities underlying Parkinson's disease (PD), its natural history, and its response to treatment. We will also describe recent applications of network analysis other neurodegenerative conditions such as Huntington's disease (HD) and Alzheimer's disease (AD).

Metabolic network mapping: a brief overview

SSM/PCA is a multivariate method designed to identify significant spatial covariance patterns in combined samples of patients and control scans [13,15,16]. This statistical model is based on the notion that small but biologically relevant signals can be detected in functional brain images after minimizing the substantive variability that exists across subjects and regions [1]. Box 1 summarizes the computational steps underlying SSM/PCA. The analysis generates a set of principal components (PCs), which are separately used to identify relevant spatial covariance patterns associated with specific disease states. The steps involved in the identification of abnormal "disease-related" spatial covariance patterns are summarized in Box 2. Because the analysis is performed on the entire sample, SSM/PCA operations are blind to subject class designation. This method of pattern detection utilizes metabolic data from the entire brain without *a priori* assumptions regarding the contributions of specific regions to the covariance patterns. Moreover, because the global mean for each subject is removed in computing the SRP before applying PCA, the resulting regional covariance patterns are not influenced by individual differences in this measure [cf. 9].

As part of the analysis, SSM/PCA also quantifies pattern expression in individual subjects (i.e. the PCA scalars or subject scores). Once a significant pattern has been identified, its expression in a given subject can be computed prospectively from that individual's scan [13,17] (Box 3). The resulting network values can discriminate between groups and/or be correlated with independent clinical, behavioral, or physiological disease descriptors. Notably, these patterns typically exhibit increased signal-to-noise with disease progression. This attribute and the

objective, data-driven features of SSM/PCA computations make this an optimal tool to evaluate abnormalities in functional brain organization in neurodegenerative disorders.

Metabolic networks in Parkinson's disease

The PD-related motor pattern

PD is a neurodegenerative disorder characterized by motor and non-motor symptoms [18]. The three cardinal motor symptoms of PD are resting tremor, bradykinesia (slowing of movement), and rigidity. Non-motor symptoms can include cognitive impairment, affective disorder, and other clinical features. Data from experimental animal models indicate that manifestations of this disease cannot be attributed to isolated dysfunction of the basal ganglia. Rather, presynaptic nigrostriatal dopamine loss is associated with broader functional abnormalities involving cortico-striato-pallido-thalamocortical (CSPTC) loops and related pathways [19]. It is therefore not surprising that network analysis of functional imaging data in PD revealed a specific abnormal spatial covariance pattern involving major elements of this circuitry [9,13]. The Parkinson's disease-related pattern (PDRP) is characterized by increased pallido-thalamic and pontine metabolic activity associated with relative reductions in premotor cortex, supplementary motor area (SMA), and in parietal association regions (Figure 1a). To date, topographically similar PDRPs have been detected in seven independent patient populations scanned using vastly different rest-state imaging techniques [20]. Comparison of these spatial patterns revealed that the subtle variations in PDRP topography (as defined by the region weights on each significant network) observed across populations stem mainly from differences in tomographic resolution (Figure 1b). PDRP expression in individual subjects has excellent test-retest reproducibility over hours to weeks [13], as well as exhibiting a consistent linear relationship with standardized motor ratings in multiple patient cohorts [21-23] (Figure 1c).

In addition, PDRP expression discriminates accurately between classical PD, atypical parkinsonian syndromes, and healthy control subjects in prospective FDG PET scans [24]. Indeed, distinctive abnormal spatial covariance patterns have been identified for multiple system atrophy (MSA) and progressive supranuclear palsy (PSP), the two most common parkinsonian “look-alike” conditions [25]. These disease-related patterns in conjunction with the PDRP provide accurate differential diagnosis of these diseases based entirely on pattern recognition [16]. Because cerebral blood flow and metabolism are tightly coupled in the absence of medication [13,26], discrimination between these groups was also achieved with PDRP measurements in scans of resting cerebral perfusion acquired by $H_2^{15}O$ PET [13], ECD SPECT [23,24], and arterial spin labeling (ASL) MRI methods [27].

The functional/anatomical basis for the PDRP spatial topography conforms well to changes in regional synaptic activity described in experimental animal models [9,14,26,28]. The metabolically active regions include both pallidal segments (elevated putamen-GPe and STN-GPi inputs), ventrolateral thalamus (elevated GPi-thalamic input), and pedunculo-pontine nucleus (elevated GPi-PPN input). Although this interpretation of PDRP covariance structure does not take into account the dynamic properties of neuronal discharges in these regions, it generally accords with traditional “box and line” models of CSPTC circuitry [19]. Indeed, this view has been recently substantiated in PD patients undergoing stereotaxic surgery for the implantation of deep brain stimulation (DBS) electrodes in the subthalamic nucleus (STN). Intraoperative recordings of spontaneous firing rates from this structure correlated closely with PDRP expression measured preoperatively with FDG PET (Figure 1d). Thus, the PDRP is linked to neuronal activity at a critical modulatory site for basal ganglia output [29].

Further demonstration of the relationship of the PDRP to abnormal network organization in PD has been provided by imaging studies of individuals undergoing therapeutic STN lesioning

(subthalamotomy) [30,31]. As expected, this procedure reduced PDRP expression on a sustained basis. Moreover, the location, magnitude, and direction of post-lesioning changes in regional metabolism conformed closely to PDRP topography, involving network areas (“nodes”) in which regional metabolism was correlated with STN firing rate[32]. Indeed, at the nodal level, changes in local metabolism following surgery were determined by network topography. Thus, post-operative metabolic decreases occurred in brain regions with positive voxel weights on the network (i.e., relatively hyperactive “red” areas). By contrast, following surgery, metabolic increases were evident in brain regions with negative voxel weights on the network (i.e., relatively underactive “blue” areas) [9]. These data indicate that PDRP expression can be modulated by focal lesions within the network, with predictable downstream effects on regional brain function. Interestingly, clinical outcome following anti-parkinsonian therapy has been found consistently to relate to the degree of PDRP suppression that was achieved by the intervention [21,31,33,34].

The PD-related cognitive pattern

Network analysis has also been used to study the cognitive changes associated with PD. The prevalence of dementia in PD has conservatively been estimated to be between 25% and 30%, and more circumscribed cognitive deficits appear many years before the onset of dementia [35,36]. Voxel-based spatial covariance analysis of metabolic imaging data from non-demented PD patients revealed a distinct pattern that was related to cognitive dysfunction [37]. This PD-related cognitive pattern (PDCP) was characterized by a covariance structure different from PDRP, with metabolic reductions mainly in medial frontal and parietal association regions, and relative increases in cerebellar cortex and dentate nuclei (Figure 2a). PDCP expression in individual subjects correlated with performance on neuropsychological tests of memory and executive functioning in an original pattern derivation cohort and in a larger prospective validation cohort (Figure 2b). Indeed, these values were found to increase stepwise in PD patients categorized by clinical criteria for mild cognitive impairment (MCI) [38] (Figure 2c). Like the PDRP, PDCP expression is highly reproducible in individual patients [37]. Importantly, although the activity of both networks increased linearly as a function of symptom duration, the rate of increase in PDCP expression was slower than that of the PDRP measured in the same subjects (Figure 2d).

Overall, these cross-sectional studies suggest that different neural systems underlie these two disease-related metabolic networks. The divergence of the PDRP and PDCP metabolic networks is particularly evident when the time course of pattern expression was assessed in individual subjects undergoing serial imaging with disease progression.

Changes in network activity with disease progression

Despite similar underlying pathology, the rate of observed clinical decline in patients with a given neurodegenerative disorder can vary considerably, even among individuals of similar baseline phenotype and disability level. Therefore, imaging biomarkers have been actively pursued as objective and potentially more accurate descriptors of disease progression. In PD, disease-related spatial covariance patterns have already provided unique information concerning the functional mechanisms at the systems level that underlie motor and cognitive deterioration [39]. In a longitudinal imaging study, patients with early stage PD were scanned at baseline, 24 months, and 48 months. Regional glucose metabolism and PDRP/PDCP network activity were quantified at each time point with FDG PET. In addition to standardized motor ratings, caudate and putamen dopamine transporter (DAT) binding was also measured at each time point as markers of presynaptic nigrostriatal dysfunction. These studies revealed that increases in PDRP expression over time occurred in association with concurrent deterioration in motor ratings and putamen DAT binding [39]. Correlations between changes in these variables were significant, albeit of modest degree. Indeed, approximately a third of

individual differences in any one of these progression biomarkers was explained by either of the two other biomarkers [20]. Therefore, rather than being interchangeable, each of these measures captures a unique feature of disease progression.

Although this longitudinal cohort involved only 15 subjects, the results were consistent with the cross-sectional results (Figure 2d) indicating that the motor and cognitive features of PD are mediated by distinct anatomical/functional pathways that develop at different rates. Moreover, when the metabolic networks were quantified on a hemispheric basis, the changes in PDRP expression on the “preclinical hemisphere” were found to precede the appearance of motor signs on the opposite body side by approximately two years [40]. This suggests that abnormal network activity can be a feature of prodromal parkinsonism, reflecting functional decompensation prior to the appearance of discernible clinical manifestations. Ongoing cross-sectional and longitudinal network studies in carriers of susceptibility genes for PD will determine whether disease-related patterns are indeed expressed preclinically, and if so, provide critical data concerning the time course of these network changes as symptoms emerge.

Network modulation with PD treatment

As mentioned above, the quantification of changes in metabolic network activity during treatment can provide an objective means of assessing the effects of therapy on brain function [21,31]. The degree of network modulation during treatment can be viewed as an *in vivo* measure of the functional changes that occur at the systems level. This strategy has recently been found useful in evaluating novel experimental interventions at early phases of translation to the clinic. It has also shed light on mechanisms underlying the side effects of otherwise effective interventions.

Dopaminergic treatment

Reductions in PDRP expression have been reported consistently during uncomplicated levodopa therapy for PD [21,41]. Indeed, these network level changes correlate with clinical improvement. In general, tight correlations (i.e. coupling) exist between network values measured in metabolic scans (i.e. FDG PET) and in scans of cerebral blood flow (i.e. H₂¹⁵O PET, Tc-99m, arterial spin-labelled MRI) from the same subjects [13,27,42]. Nonetheless, a recent study demonstrated significant flow-metabolism dissociation in PDRP expression during levodopa administration [26] (Figure 3a, *top*). This phenomenon, striking at the network level, was evident in individuals without complications of levodopa therapy. Notably, the dissociation of flow and metabolism with levodopa therapy was most pronounced in subjects with treatment-induced dyskinesias during the scans. Regional analysis revealed that the dissociation between the synaptic and neurovascular effects of dopaminergic therapy was caused by the local vasoactive effects of dopamine synthesized in areas rich in dopa decarboxylase (DDC) within the PDRP network [26,42]. The precise role of flow-metabolism dissociation at both the regional and network levels in the pathogenesis of levodopa-induced dyskinesias is not known. Further research is needed to determine whether dissociation is also present with other forms of dopaminergic pharmacotherapy, and whether this effect can be selectively mitigated with novel antidyskinetic agents.

This study also demonstrated that coupling in PDCP regions is maintained during treatment with either levodopa infusion or STN stimulation (Figure 3a, *bottom*). Moreover, the effects of levodopa treatment on cognition have been found recently to involve non-linear changes in prefrontal deactivation during task performance [43]. The demonstration of analogous treatment-mediated changes in PDCP expression will help substantiate this imaging measure as an objective outcome variable in clinical trials targeting the cognitive manifestations of PD.

Stereotaxic interventions

Direct neurosurgical modulation of abnormal STN activity has become an alternative to dopaminergic pharmacotherapy in patients with advanced motor symptoms. Indeed, the functional mechanisms mediating clinical benefit are thought to be similar for STN DBS and levodopa therapy [44]. The findings of significant PDRP modulation with both interventions support this contention at the network level [21] (Figure 3a). Interestingly, the effects of levodopa and STN DBS on PDRP expression were not additive [21]. Data from subjects treated with both therapies revealed that it is unlikely for combination therapy to lower network activity beyond the natural “floor” determined by therapeutic STN ablation. Similarly, a recent study [45] addressed the effects of STN “microlesion” on PDRP expression, representing the change in network activity that occurs subsequent to STN electrode insertion without stimulation. Significant regional metabolic effects of electrode implantation were seen in the putamen, GP, and ventral thalamus, areas receiving direct or indirect input from the STN [32]. The magnitude of these regional changes was smaller than that seen with therapeutic STN lesioning. Moreover, STN microlesion was not associated with significant PDRP suppression or clinical improvement, as has been observed following subthalamotomy or STN stimulation [21,30, 31]. These findings suggest that upper and lower boundaries exist for therapeutic PDRP modulation, defining the limits of the functional changes that can be achieved by surgery in this region. Whether interventions at other key PDRP nodes such as the pedunculopontine nucleus (PPN) [46,47] can further reduce network activity is a topic of ongoing investigation.

Gene therapy

Gene therapy is a novel form of treatment for neurodegenerative diseases, including PD [48]. However, past concerns for safety prompted careful study design and assessment of safety and tolerability in clinical trials. Phase I trials for safety use small unblinded patient cohorts that are poorly suited for the objective assessment of clinical efficacy. One method to assess treatment response under such conditions is to use disease-related metabolic networks like PDRP as *in vivo* biomarkers [33]. This approach was used in a phase I clinical trial of STN gene therapy for advanced PD using adeno-associated virus (AAV) borne glutamic acid decarboxylase (GAD) [49]. FDG PET was performed on 12 patients with advanced PD before, 6 months after, and 12 months after unilateral gene therapy. The activity of the PDRP on the side of STN AAV-GAD infusion was significantly reduced at 6 months with sustained reduction at 12 months (Figure 3b, *top*). Indeed, changes in PDRP expression correlate with improvement in the clinical motor ratings [33]. By contrast, PDCP expression did not change after gene transfer (Figure 3b, *bottom*), consistent with absence of cognitive changes after surgery. Thus, the quantification of treatment-mediated changes in pattern expression in individual subjects provides an objective means of assessing response, even in unblinded early phase trial designs. This unique information may be particularly useful in developing new treatment strategies for PD and other neurodegenerative diseases.

Network analysis in other neurodegenerative disorders

Spatial covariance mapping techniques have recently been applied to other neurodegenerative disorders like Huntington's disease (HD) and Alzheimer's disease (AD), for which therapeutic options are rather limited. In both these disorders, network analysis has already yielded specific reproducible disease-related covariance patterns that can potentially be used in clinical investigation. As in PD, these patterns provide unique systems-based functional data to complement other imaging methodologies.

Huntington's disease

Huntington's disease (HD) is an autosomal dominant neurodegenerative disorder characterized by progressive development of involuntary movements and cognitive abnormalities. Genetic

testing is readily available to identify premanifest gene carriers among at risk individuals. Nonetheless, there are few validated methods to assess the progression of disease before clinical onset. Indeed, such tools will prove critical for the evaluation of drugs to delay or prevent phenocconversion. Spatial covariance analysis of metabolic scans from preclinical HD carriers revealed a specific, reproducible disease-related pattern (HDRP) characterized by relative hypometabolism of the caudate, putamen and mesial temporal cortex with relative metabolic increases in the occipital cortex [50] (Figure 4a). In a 44 month longitudinal study of mutation carriers, pattern expression increased in the early premanifest disease, but declined as the subjects approached symptom onset. These network changes did not correlate with contemporaneous PET measurements of striatal D2 receptor availability, an index of the integrity of the projection neurons that are preferentially lost in HD. The non-linear trajectory of HDRP progression in the preclinical period suggests the existence of a network level compensatory mechanism that is progressively lost as symptom onset nears. In fact, post-hoc analysis of the time course data from each node of this network revealed that the ventrolateral thalamus is likely to be the locus of this compensatory process [50]. Further longitudinal studies in premanifest HD gene carriers are being conducted to better delineate these changes at both the regional and network levels. Moreover, new computational approaches to spatial covariance mapping using within-subject rather than cross-sectional designs [6] are likely to provide more accurate descriptors of preclinical disease progression in HD gene carriers [51].

Alzheimer's disease

AD is the most common neurodegenerative cause of cognitive impairment in the aging population. The introduction of radiotracer imaging to quantify the regional accumulation of fibrillated β -amyloid has had a major impact on the clinical investigation of AD [52,53]. A recent longitudinal PET study of early AD patients scanned with [^{11}C] PIB for β -amyloid and with FDG PET revealed that regional changes differed for the two imaging approaches, with the former reaching a plateau while the latter exhibited significant reduction over time [54]. In this study, changes in regional synaptic activity captured by FDG PET were also relatively better correlated with cognitive deterioration during a two year period of follow-up. Given that the topography of AD cortical pathology is stereotyped [55], recent efforts have focused on using network mapping techniques to identify disease-related patterns in brain imaging data from patients with AD and other neurodegenerative disorders of cognition. Indeed, this approach has been recently applied to resting state blood oxygen level-dependent (BOLD) signal fMRI to demonstrate that discrete large scale functional/anatomical networks are the targets of specific neurodegenerative processes [56].

Spatial covariance analysis of rest state metabolic images has been used to delineate abnormal functional patterns that are characteristic of AD. In a recent multicenter FDG PET study [15], SSM/PCA identified a robust disease-related metabolic pattern, which was replicated in five independent samples of patients and controls. In a parallel study, a topographically similar AD-related spatial covariance pattern (Figure 4b) was identified in ASL MRI scans from a small cohort of mild AD patients and controls [57]. These studies highlight the potential role of such patterns as network biomarkers for AD and other disorders. That said, further validation steps to assess the reliability of these measures between imaging session, as well as long term longitudinal follow-up studies, are required before they can be used to evaluate new AD therapies. A network-based classification system analogous to that developed for parkinsonian disorders [16] will also contribute to the clinical investigation of these disorders. Moreover, this approach is apt to be particularly useful in studying individuals carrying susceptibility genes for AD, in whom stereotyped metabolic abnormalities have been found to be present [58,59].

Conclusions and future directions

In this review, we show how new voxel-based network methods can be applied to rest state metabolic imaging data to study changes in brain function in neurodegenerative disease. The consistent disease-related network abnormalities observed using this approach reflect stereotyped changes in the functional interactions of discrete sets of brain regions in these disorders. These unique spatial patterns can be quantified, and if validated, used as biomarkers to measure disease progression and treatment effects at the system level. Indeed, the degree of network modulation observed with a novel intervention can be used as an objective measure of treatment efficacy during early phase clinical testing. Lastly, these methods may be particularly useful in elucidating compensatory mechanisms in inherited neurodegenerative diseases. Ongoing improvements in network modelling algorithms are likely to further expand the role of functional imaging in the investigation of new treatment strategies for these disorders.

Box 1. Spatial Covariance Analysis: Basic Computational Steps

- Each subject's image is spatially normalized so that functional activity measurements at different locations in the original image are mapped to a standard stereotaxic coordinate system.
- The scan data from each subject are represented in matrix form such that the voxels form a continuous row vector and each column represents a particular voxel in the standard space. For cross-sectional analysis, the data matrix consists of the images from two or more groups. Each data entry into the matrix is logarithmically transformed.
- Row averages are subtracted so that voxel values represent within-subject derivations from the mean of the subject log image. Columns are then averaged to create a characteristic mean image vector for the group. The column averages are subtracted from the individual matrix entries to produce a matrix of “residual” images termed the subject residual profile (SRP). This represents the matrix of deviations from the subject and group means.
- A covariance matrix is constructed from the subject residual profiles by computing the covariance between each pair of SRP matrix rows.
- PCA is applied to the subject by subject covariance matrix resulting in subject score vectors with associated eigenvalues. Score vectors are weighted by the square roots of their eigenvalues. The transpose SRP matrix is then multiplied by the score matrix to derive an array of voxel pattern vectors, i.e., the respective PCs. The percent of the total variance accounted for by each PC corresponds to the relative size of its eigenvalues. See Figure I.

Box 2. Identification of Significant Disease-Related Spatial Covariance Patterns

- The major PCs and the corresponding subject scores undergo further analysis to identify specific disease-related patterns. This analysis is limited to the first several PCs that cumulatively accounts for the top 50% of the subject $\times$ voxel variance.
- Subject scores for these PCs are entered singly and in all possible combinations into a series of logistic regression models, with the group (patients versus controls) as the dependent variable and the subject scores for the PCs as the independent variables in each model. The Akaike Information Criterion (AIC) [62] is computed

for each of these models and compared across models. The model with the lowest AIC value [63] is selected as the one that best distinguishes between the patient and control groups [64].

- The p-value of the likelihood ratio test for this model is used to indicate the level of discrimination between the two groups. Patterns were considered “disease-related” for $p < 0.001$ [65].
- The PC(s) selected in the best model are then used in linear combination to yield a single disease-related covariance pattern. Voxel weights at the local maxima are Z-thresholded at 3.61, corresponding to significant ($p \leq 0.001$) contributions to whole brain network activity at peak voxel and further tested for reliability using bootstrap resampling [66].

Box 3. Prospective Quantification of Pattern Expression: Single Case Analysis

- Once identified, the expression of disease-related patterns can be quantified in prospective cohorts. This is achieved on an individual case basis by multiplying the subject SRP vector by the pattern vector (i.e., the dot product of the two vectors). For these computations, the SRP vector is determined by subtracting from each voxel the subject mean log value followed by the predetermined “reference” group mean for the corresponding voxel.
- Subject scores for the pattern(s) are z-transformed with respect to the original group of patients and controls used to identify the pattern. The z-scores are offset so that the original control group has a mean value of zero. See Figure II.

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Figure 1. Parkinson's disease-related pattern: validation and correlates

(a) Parkinson's disease-related pattern (PDRP). This motor-related metabolic spatial covariance pattern is characterized by hypermetabolism in the thalamus, globus pallidus (GP), pons, and primary motor cortex, associated with relative metabolic reductions in the lateral premotor (PMC) and posterior parietal areas [13]. [In the representative slices, relative metabolic increases are displayed in red; relative metabolic decreases are displayed in blue. Slices were overlaid on a standard MRI brain template.] **(b)** Cluster analysis of PD-related regional topographies identified in seven independent combined groups of PD patients and healthy subjects (Populations A-G) who underwent metabolic imaging studies on different tomographs. Region weights for the disease-related covariance patterns (*rows*) were similar across populations ($r > 0.7$, $p < 0.001$). Consistent increases in network-related metabolic activity (*red*) were present in the putamen/globus pallidus, thalamus, and cerebellum; relative decreases (*green*) were present in the lateral premotor/prefrontal cortex and parietal association regions. Of note, the pattern topographies for the various populations (*columns*) clustered according to the resolution of the respective tomographs, such that similar PDRPs were discerned for instruments of low (Populations B, F: 12 mm FWHM), medium (Populations A, C, E: 8 mm FWHM), and high (Populations D, G: 4 mm FWHM) resolution. [A region-of-interest (ROI) approach was used in which a standard brain template was used to measure local metabolic activity in 30 anatomically pre-specified areas. For each population, a significant disease-related pattern was identified using subject scores for the first two principal components (singly

or in linear combination) such that the two groups were separated at $p < 0.001$, discriminant analysis. In this display, the region weights for each significant pattern, reflecting the contribution of local metabolic activity in each ROI to overall pattern expression [4,9,13], were displayed on a heat map based on hierarchical clustering analysis [60]. Cluster trees were created using a complete linkage method with the Pearson correlation coefficient (uncentered) as the similarity measure. Region weights for the PDRPs from each population were presented elsewhere [20]]. **(c)** PDRP expression correlates with Unified Parkinson's Disease Rating Scale (UPDRS) motor scores from a combined group of three independent PD populations ($n=65$; $r=0.68$, $p < 0.001$) [21,22,61]. These correlations were also significant within each of the individual patient cohorts (*circles*: $n=27$; $r=0.66$; $p < 0.001$; *squares*: $n=15$; $r=0.65$, $p < 0.01$; *triangles*: $n=23$; $r=0.76$, $p < 0.001$). **(d)** PDRP expression correlates with intraoperative measurements of spontaneous subthalamic nucleus (STN) firing rate ($n=17$; $r=0.76$, $p < 0.007$) recorded in PD patients undergoing the implantation of deep brain stimulation (DBS) electrodes [32]. The regression line includes corrections for individual differences in disease duration and motor ratings across the subjects. [*Brain* 131 (Pt 5), 1373-1380 Copyright © 2008 of Oxford University Press]

Figure 2. Parkinson's disease-related cognitive pattern: validation and correlates

(a) Parkinson's disease-related cognitive pattern (PDCP). This cognition-related metabolic spatial covariance pattern is characterized by hypometabolism of dorsolateral prefrontal cortex (PMC), rostral supplementary motor area (preSMA), precuneus, and posterior parietal regions, associated with relative metabolic increases in the cerebellum [37]. [In the representative slices, relative metabolic increases are displayed in red; relative metabolic decreases are displayed in blue. Slices were overlaid on a standard MRI brain template.] (b) PDCP expression correlates with performance on neuropsychological tests of memory and executive functioning in non-demented PD patients. For the California Verbal Learning Test: Sum 1 to 5 (CVLT sum), this correlation was significant for the entire cohort ($n=56$; $r=-0.67$, $p < 0.001$), as well as for the original group used for pattern derivation (*circles*; $n=15$; $r=-0.71$, $p=0.003$) and in two prospective validation groups (*squares*; $n=25$; $r=-0.53$, $p=0.007$; *triangles*; $n=16$; $r=-0.80$, $p<0.001$) [37]. [Revised version of Neurology 34(2):714-723 Copyright © 2007 by AAN Enterprises, Inc.] (c) Bar graph of PDCP expression (mean $\pm$ SE) in PD patients with dementia (PDD), multiple domain mild cognitive impairment (MCI(m)), single domain mild cognitive impairment (MCI(s)), PD patients without mild cognitive impairment (MCI(-)), and in healthy control subjects. There was a significant difference in PDCP expression across the patient and control groups ($F(4,70)=8.87$, $p<0.001$; one-way ANOVA) and among the PD groups ($F(3,56)=4.84$; $p<0.005$), with higher expression in the PDD and MCI(m) cohorts compared to the MCI

(-) cohort ($p < 0.03$; Tukey-Kramer HSD). For each PD group, PDCP expression was separately compared to healthy control values using Student *t*-tests. The asterisks denote significant increases in network activity relative to controls (* $p < 0.05$, ** $p < 0.005$, *** $p < 0.0001$) in all PD categories including MCI(-). [Revised version of Neurology 70(16 Pt 2):1470-1477 Copyright © 2008 by AAN Enterprises, Inc.] **(d)** Progressive increases in PDRP (*circles*) and PDCP (*triangles*) expression as a function of disease duration. Regression analysis showed that activity of each of the two networks increased linearly over time ($p < 0.0001$). However, the rate of increase in pattern expression differed over time for the two networks ($p < 0.02$, interaction effect), with slower progression of PDCP activity relative to the PDRP.

Figure 3. PD-related metabolic networks: treatment effects

(a) Bar graph illustrating treatment-mediated changes (mean $\pm$ SE) in PDRP and PDCP expression measured in scans of cerebral metabolic rate for glucose (CMR; filled bars) and cerebral blood flow (CBF; open bars) [26]. *Top*: There was a significant dissociation in PDRP expression between the CMR and CBF treatment responses ($p < 0.001$) with levodopa (LD; left), but not with subthalamic nucleus deep brain stimulation (STN DBS; middle) or in PD controls (PD CTRL; right) undergoing repeat imaging on stable doses of dopaminergic medication [26]. *Bottom*: No significant CMR or CBF responses in PDCP expression were found with either treatment (levodopa or STN DBS) in the same patients. [*J. Neurosci.* 28, 4201-4209 Copyright © 2008 by the Society for Neuroscience] (b) Changes in PDRP and PDCP expression over time for the operated (filled circles) and unoperated (open circles) hemispheres following subthalamic (STN) gene therapy [33]. *Top*: There was a significant difference ($p < 0.002$) in the time course of PDRP expression between the two hemispheres. Network activity declined during the first six months and then increased over the subsequent six months in the operated hemisphere. By contrast, network activity in the unoperated hemisphere increased continuously over the entire 12 months following surgery. *Bottom*: There was no change in PDCP activity over time in either of the two hemispheres following gene therapy ($p = 0.72$). [The dashed line represents one standard error above the normal mean value of zero for each pattern]. [*Proc. Natl. Acad. Sci. U. S. A.* 104, 19559-19564 Copyright © 2007 National Academy of Sciences, U.S.A.]

Figure 4. Spatial covariance patterns for Huntington's and Alzheimer's diseases

(a) Huntington's disease (HD)-related spatial covariance pattern. This pattern was characterized by relative metabolic decreases in the striatum and cingulate cortex, associated with relative increases in the ventral thalamus, cerebellum, motor cortex, and occipital lobe [50]. [In the representative slices, relative metabolic increases are displayed in red; relative metabolic decreases are displayed in blue. Slices were overlaid on a standard MRI brain template. [*Brain* 130 (Pt 11), 2858-2867 Copyright © 2007 of Oxford University Press] **(b)** Alzheimer's disease (AD)-related spatial covariance pattern identified using continuous arterial spin labeling (CASL) MRI [57]. Areas displayed with network-related CBF reductions in AD relative to healthy controls were found to be reliable by bootstrap resampling. Most significant areas within the network were located in the vicinity of the parahippocampal gyrus. No areas with positive region weights on the pattern (i.e., relative CBF increases in the AD subjects) were discerned [Reprinted by permission from Macmillan Publishers Ltd: *J Cereb Blood Flow Metab* 28(4): 725-736 Copyright © 2007].

Figure I (Box 1).
Metabolic Network Analysis: Computational Diagram

Figure I (Box 3).
Metabolic Network Expression: Computational Diagram