Chapter 4

Computational prediction of changes in brain metabolic fluxes from mRNA expression in Parkinson’s disease

Farahaniza Supandi
Johannes HGM van Beek

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Abstract

Background: Parkinson’s disease is one of the most widespread neurodegenerative disorders and affects brain metabolism. Although changes in gene expression during the disease are often measured, it is difficult to predict metabolic fluxes from gene expression data. Here we apply a new computational method to predict metabolic flux changes from post-mortem gene expression measurements in Parkinson’s disease (PD) brain.

Results: We use a network model of central metabolism and optimize the correspondence between relative changes in fluxes and in gene expression, taking flux balance and reaction reversibility constraints into account. To this end we apply the Least-squares with Equalities and Inequalities algorithm integrated with Flux Balance Analysis (Lsei-FBA). We predict for PD (1) decreases in glycolytic rate and oxygen consumption and an increase in lactate production in brain cortex that correspond with measurements (2) relative flux decreases in ATP synthesis, in the malate-aspartate shuttle and midway in the TCA cycle that are substantially larger than the decreases in glucose uptake in the substantia nigra, dopaminergic neurons and most other brain regions (3) shifts in redox shuttles between cytosol and mitochondria (4) in contrast to Alzheimer’s disease: little activation of the gamma-aminobutyric acid shunt pathway in compensation for decreased alpha-ketoglutarate dehydrogenase activity (5) in the globus pallidus internus, metabolic fluxes are predicted to be increased, reflecting increased functional activity. During PD, decreases in brain ATP synthesis may be substantially larger than suggested by the reduced glucose uptake.

Conclusion: The results from our method indicate that prediction of changes in metabolic fluxes from gene expression data is feasible, at least for Parkinson’s disease. The computational predictions correspond with independent measurements of brain metabolism where available. The new computational method enables to extrapolate predictions to metabolic pathways in neurons and brain regions where accurate measurements of metabolic fluxes are not yet available.

Keywords: Metabolic fluxes, metabolic network, brain metabolism, Parkinson’s disease, neurodegeneration
Background

Many human diseases are associated with changes in central metabolism. Changes in expression of metabolic genes during disease are often measured, even in small anatomical regions or specific cell types. Unfortunately it was considered difficult to predict changes in metabolic fluxes from the gene expression changes (Daran-Lapujade et al. 2007). However, recently it was reported that metabolic fluxes in yeast can be meaningfully predicted based on absolute gene expression in yeast (Lee et al. 2012). Here we apply an approach to predict changes in metabolic flux distribution during disease from changes in gene expression in human tissue. Our approach, termed Lsei-FBA, was described and demonstrated on one data set for Alzheimer’s disease recently (Gavai et al. 2015). It is not meant to be an exact calculation based on enzyme kinetic equations, enzyme activities and metabolite concentrations, but a bioinformatic prediction of changes at the network level based on the tendencies suggested by gene expression changes. In this regard it builds on the idea that it is possible to predict changed activity of biological pathways from associated gene expression changes, extending this in the direction of rough quantitative predictions for flux distributions in metabolic networks. The Lsei-FBA approach is applied here to gene expression data for Parkinson’s disease.

Parkinson’s disease (PD) is one of the most widespread neurodegenerative disorders. PD is characterized among others by movement disorder, rigidity and tremor caused by the loss of dopaminergic neurons in the substantia nigra pars compacta (SNC) of the brain. Although several genes have been identified in familial cases and by genome wide association analysis, the mechanisms for the PD progression are largely unknown. Damage to the mitochondria resulting in failure to generate energy possibly contributes to PD (Banerjee et al. 2009; Schapira 2010). Several gene products linked to PD show mitochondrial localizations. Mitochondrial dysfunction has also been implicated in other neurodegenerative diseases such as Alzheimer’s disease (AD), Huntington’s disease (HD) and Amyotrophic Lateral Sclerosis (ALS) (Lin & Beal 2006).

PD is often associated with disturbed mitochondrial function in the neurons in the SNC which are the most conspicuous target of the disease. Decrease in complex I activity in the electron transport chain (ETC) during PD has been measured in the substantia nigra (Schapira et al. 1989) and frontal cortex (Parker et al. 2008) of post-mortem brain. Reduction of other ETC complexes, namely complex II, III and IV has also been reported for the substantia nigra, platelets and muscle (reviewed in Banerjee et al. 2009; Zhu & Chu 2010).

Statistical analysis of gene expression also suggests that mitochondrial electron transport and glucose metabolism in the SNC and other brain regions are affected (Zheng et al. 2010). However, the pattern and the magnitude of the changes in metabolic flux distribution are unknown. Accurate measurements of metabolic fluxes in
the small brain regions and cell types targeted by PD are presently impossible. Measurements of oxygen and glucose uptake with positron emission tomography (PET) in PD patients have been done in larger brain regions (Borghammer et al. 2010), and increased lactate accumulation has been measured with NMR spectroscopy (Henchcliffe et al. 2008). Because it is difficult to measure metabolic reaction rates directly in small brain regions or in specific cell types, it is useful to predict redistribution of metabolism from mRNA expression measured in the small regions affected by PD, such as the SNc and specifically in dopaminergic neurons.

The Lsei-FBA approach to predict changes in central energy metabolism during PD start with establishing the metabolic flux distribution in the normal brain based on measured data for the uptake and production of metabolites in healthy human brain (Gavai et al. 2015). This data is analysed using flux balance analysis (FBA) of a network model of central energy metabolism to predict the flux distribution in normal brain. The change in flux distribution during PD is then calculated based on our assumption that, on average, the flux carried by each enzyme tends to change proportionally to the change in mRNA expression between controls and PD patients. Note that we do not assume that every reaction rate changes in proportion to the gene expression level, but that on average the reaction fluxes tend to follow gene expression. In the next step, also for the diseased state, we maintain the assumption of balance of fluxes in the metabolic network because metabolites that are not exchanged between brain tissue and blood cannot keep on accumulating steadily during chronic disease and their production and consumption must therefore be approximately balanced. The changes in mRNA expression provide a first rough prediction of the change in metabolic fluxes based on the assumption that the relative change in gene expression and in metabolic flux for the genes tend to correspond. This initial rough estimate is refined by using the consistency and balance of fluxes in the metabolic network as additional constraints. The assumption of proportionality between gene expression and enzymatic flux, at least on average, will be discussed below.

The final prediction of metabolic fluxes in the network is subject to 1) flux balance for metabolites which are not exchanged between brain and blood 2) restriction to forward flux through irreversible reactions 3) maximization of correspondence between relative changes in mRNA expression and changes in fluxes. We include expression datasets from the SNc and from laser captured microdissected (LCM) dopaminergic neurons, in which neuronal damage usually occurs most prominently during PD. These SNc measurements are compared with other brain regions that show abundant Lewy bodies (LB) in PD without neuronal loss, such as frontal cortex, prefrontal cortex Brodmann area 9 (BA9) and basal ganglia structures. A statistical meta-analysis at the gene-set level of these datasets (Zheng et al. 2010) was already done, showing significant changes in mitochondrial electron transport and glucose metabolism, was already reported and is not repeated here. In the present study we report quantitative predictions of the changes in the distribution of fluxes in
central energy metabolism in these specific brain regions and in dopaminergic neurons in PD.

Methods

Metabolic model reconstruction for brain metabolism

A metabolic reaction network was constructed consisting of the major pathways representing central carbon and energy metabolism in the brain. The rationale for using this model and an extensive comparison with a larger model of brain metabolism has been described in Gavai et al. (2015). Metabolites and enzymatic reactions were distributed over the extracellular, cytosolic and mitochondrial compartments. In summary, the pathways include glycolysis, pentose phosphate pathway (PPP), TCA cycle, oxidative phosphorylation (OxPhos), reducing equivalent shuttling mechanisms, gamma-aminobutyric acid (GABA) shunt and transport of metabolites across the membranes which separate the compartments. We updated this model by adding glutamate-glutamine cycle, pyruvate carboxylase reaction and ammonium transport across the mitochondrial membrane. The selected reactions were imported from the BiGG database (Schellenberger et al. 2010). Complete lists of the reactions in the network along with the lists of metabolite are given in Supplementary Tables S1 and S2. The Supplementary Figure S1 shows the network scheme.

Analysis of mRNA expression data

Datasets containing the CEL files containing gene expression data of individual post-mortem brain samples for neuropathologically confirmed PD patients and normal controls from the same study were downloaded from the Gene Expression Omnibus (GEO) database (Edgar et al. 2002) and the National Brain Databank (NBD; http://national_databank.mclean.harvard.edu/brainbank/Main) and are summarized in Supplementary Table S3. The datasets are given in (Grünblatt et al. 2004; Hauser et al. 2005; Zhang et al. 2005; Moran et al. 2006; Papapetropoulos et al. 2006; Vogt et al. 2006; Scherzer et al. 2007; Zheng et al. 2010). The dataset from Cantuti-Castelvetri et al. (Cantuti-Castelvetri et al. 2007) (GEO accession GSE24378) was excluded from the flux analysis presented in this paper for reasons given in the Discussion, although the result of the Lsei-FBA analysis is given separately in Supplementary Table S4.

All Affymetrix CEL files were pre-processed and normalized in the R programming environment using the RMA method (Irizarry et al. 2003). Log2 transformed values were used to calculate differences in expression levels of PD patients against the healthy controls.
Mapping of expression data on a pathway map

Based on the reactions in our network, a visual map was drawn incorporating pathways downloaded from KEGG (Kanehisa & Goto 2000) and WikiPathways (Kelder et al. 2009) and modified manually in the pathway visualization tool PathVisio (van Iersel et al. 2008). Log2 transformed gene expression data were mapped onto the pathway map using the visualization options within the PathVisio tool.

Analysis of flux distribution

A list of reaction equations was prepared according to the reaction list in the BiGG database (Supplementary Table S1). The metabolic system is assumed to be in steady state. Substrate uptake measurements for the healthy elderly (55-65 years) human brain were taken from (Lying-Tunell et al. 1980), which reported the uptake rates of glucose, and release of lactate, glutamine and pyruvate for the brain to be 0.203, -0.0092, -0.011 and -0.0024 μmol g wet brain⁻¹ min⁻¹ respectively. A small flux is measured in the PPP in the normal brain, which amounts to 6.9% of glycolysis (Dusick et al. 2007). Pyruvate carboxylation and glutamate-glutamine cycling fluxes amount to 13% and 62% of the value of the total glucose uptake in the brain, respectively (Hyder et al. 2006) while the GABA shunt flux is 32% of the glucose uptake value (Patel et al. 2005). These values are used as constraints in the model.

Flux balance analysis for the normal brain was done assuming a cost function which maximizes ATP synthesis. The rationale for this assumption was discussed extensively in Gavai et al. (2015). Assuming maximal growth, which is often used for flux balance analysis of bacterial metabolism, is inappropriate because brain tissue in adults does not show net growth; some material may be turned over, but the overall change in mass is negligible. Because ATP synthesis in the mitochondria is driven by the proton motive force across the inner membrane, the balance of mitochondrial protons determines the synthesis of ATP. Internal metabolites which are not exchanged are assumed to be balanced, which means that the fluxes producing and consuming the internal metabolite sum up to zero, i.e. flux balance is enforced. The flux distribution in the healthy brain was subsequently solved using the linear programming routine Linp from the package LiM (Soetaert & van Oevelen 2009) for the R programming environment.

The flux distribution in the PD patients is estimated using the Lsei-FBA method, based on the changes in gene expression data and the flux distribution in normal brain (Gavai et al. 2015). In brief, for each reaction, the average fold change from controls was computed for the expression of each gene associated with the biochemical reactions in the model (Supplementary Figure S2). The fold change for gene expression in the PD patients times the flux estimated for the associated biochemical reaction for the healthy brain yields the initial rough estimate of the flux for every reaction in the model.
In the next step, flux estimation was refined based on flux balance in the model (Supplementary Figure S1). Under the assumption of absolute flux balance of the internal metabolites in the model and of zero backflux for the irreversible reactions as given in Supplementary Table S1, a cost function was minimized consisting of the sum of the squared deviations between final estimated flux and initial rough estimate of the flux as calculated above.

The equations of this problem of least squares with equalities (balanced fluxes) and inequalities (irreversible reactions) were solved using the least squares with equality and inequality conditions (lsei) method from the limSolve package (Soetaert et al. 2009). This method, termed Lsei-FBA, has been described in detail in (Gavai et al. 2015) and is a special case of quadratic programming. The steps describing the method are summarized in Figure 1.

**Figure 1. Flow diagram for the Lsei-FBA approach**

Flow diagram of the steps to predict metabolic fluxes for the normal brain (green boxes) and for diseased brain based on gene expression data (red boxes) described in the Methods section. For the normal brain, the flux distribution was computed from a reconstructed model of cerebral central carbon metabolism. For the diseased brain, mRNA gene expression fold changes were first computed for patients with Parkinson’s disease (PD) versus controls. An initial flux estimate is computed for each reaction in the network by multiplying gene expression fold changes with the FBA flux predictions for the normal brain. The final flux estimate is solved subject to i) forward flux in irreversible reactions, ii) maintaining the balance of fluxes during chronic disease and iii) a least squares cost function to minimize the sum of the squared deviation between the initial and the final flux estimate.
Statistical test for change of flux during disease

The difference in flux was calculated for $n=8$ studies of gene expression in the whole substantia nigra or dopaminergic neurons from that region. The significance of the difference in flux between normal controls and the eight predictions (whole SNc and dopaminergic neurons) was tested using a one-sample $t$-test ($p < 0.05$). To control for multiple comparisons the Family-wise Type 1 error (FWER) was calculated. Because the flux in a sequence of reactions that contains no side-branch is absolutely the same and therefore completely dependent, only one $t$-test was done per each group of such fluxes, e.g. $R_{GLCt1r}$ and $R_{HEX1}$ form one group, $R_{PGK}$, $R_{PGM}$, $R_{ENO}$ and $R_{PYK}$ form a group, etc. For comparison the test was also calculated for the six SNc study groups, excluding the dopaminergic neurons.

Results

PD gene expression pattern across brain regions

Fold changes of mRNA expression of patients with PD against their healthy controls are shown mapped on the reaction network in the substantia nigra and dopaminergic neurons in Supplementary Figure S2A (SN datasets), while fold changes for the internal segment of the globus pallidus (GPi), putamen, frontal cortex, cerebellum, blood and lymphoblastoid cells are shown in Supplementary Figure S2B (non-SN datasets). Downregulated genes are shown in green, upregulated genes in red.

The SN data for the expression in the glycolytic pathway shows mostly downregulation except for the hexokinases HK2 and HK3, phosphofructokinase PFKL and aldolase ALDOB genes. The solute carriers for glucose and lactate in the cell membrane tend to show upregulation. The expression changes in the pentose phosphate pathway (PPP) are small and mixed. Pathways in the mitochondria are generally downregulated, including the TCA cycle, oxidative phosphorylation and transfer of reducing equivalents across the mitochondrial membrane. However, the pyruvate dehydrogenase kinase PDK4, which participates in the regulation of pyruvate dehydrogenase activity, tends to show upregulation. Interestingly, the expression of mitochondrially encoded genes (mtDNA) in the electron transport chain (ETC) such as ND1, ND2, ND3, ND4, ND4L, ND5, ND6, CYTB, COX1, COX2, COX3, ATP6 and ATP8 are increased.

Outside the substantia nigra, transcription level changes are in general similar as in the SN datasets, with the GPi region (GSE20146) forming a clear exception. The GPi shows upregulation in most glycolytic genes while TCA cycle and oxidative phosphorylation genes are not downregulated and even show a tendency of slight upregulation (Supplementary Figure S2B).
Predicted flux distribution in the healthy brain

Measurements show that 0.203 µmol g brain (wet)$^{-1}$ min$^{-1}$ of glucose is taken up in the normal brain of elderly people, and a small amount of lactate is excreted under baseline conditions (Lying-Tunell et al. 1980). Based on this metabolic input, we estimate that 5.39 µmol g brain (wet)$^{-1}$ min$^{-1}$ ATP is produced in the brain mitochondria. The predicted flux distribution is given in Figure 2A. The malate-aspartate shuttle transports reducing equivalents into the mitochondria. The glycerol phosphate shuttle is predicted to be inactive.

To investigate if the FBA yields unique values, we performed a Flux Variability Analysis (FVA) (Mahadevan & Schilling 2003), to estimate the feasible minimum and maximum of all fluxes. The FVA proved that the fluxes calculated give indeed a unique solution for this model (data not shown).

Predicted flux distribution during Parkinson’s disease

We now predict changes in the metabolic flux distribution from the changes in gene expression data between the normal brain and PD. In most cases, fluxes are decreased from control based on the substantia nigra and dopaminergic neuron gene expression datasets. A full list of flux predictions for the substantia nigra and its dopaminergic neurons is given in Supplementary Table S4. The glycolytic flux is predicted to be reduced by 10% on average during PD, while flux into the TCA cycle decreases by 12% and 6 percent of pyruvate influx is used to produce lactate (Figure 2B). The malate-aspartate shuttle carrying reducing equivalents into the mitochondria is reduced by 18%. In addition, the glycerol phosphate shuttle becomes slightly active. Total export of ATP from the mitochondria decreases by 20% to 4.307 µmol g wet brain$^{-1}$ min$^{-1}$. In PD, on average, the GABA shunt is increased slightly (about 10%), partially compensating for the measured reduction in alpha ketoglutarate dehydrogenase (AKGDH) expression, whose flux is reduced by 20%. It is striking that the modest decrease in glucose uptake leads to an appreciably larger relative decrease in ATP production.

Flux changes in the frontal cortex, BA9, putamen and cerebellum during PD follow the same pattern as in the SN although the changes in these regions tend to be slightly smaller compared to the SN regions. Their average is given in Figure 2C and the predicted flux distribution for non-SN brain regions is given in the Supplementary Table S5. The change in fluxes in the globus pallidus internus is quite different from the substantia nigra and all other regions. In the GPi, increased flux from the normal condition is predicted in most of the pathways: glycolysis increased by 16%, lactate production is (17% of glycolytic flux), malate-aspartate shuttle is 5% higher), TCA cycle and OxPhos are on average 5% higher (Figure 2D). An increase in ATP production to 5.35 µmol g wet brain$^{-1}$ min$^{-1}$ through oxidative phosphorylation is predicted, utilizing 1.17 µmol g wet brain$^{-1}$ min$^{-1}$ of oxygen. In this case the AKGDH flux is not reduced as in
other brain regions, but slightly increased while the GABA shunt, AKGDH’s potential bypass, is slightly reduced.

Figure 2. Flux distribution in healthy brain and during Parkinson’s disease

Flux distribution in healthy brain (A) and during Parkinson’s disease in the substantia nigra (B, average from seven SN data sets), averaged value for frontal cortex, BA9, putamen and cerebellum (C) and value for globus pallidus internus region (D) in µmol g (wet) brain⁻¹ min⁻¹. Black numbers, flux during normal condition; green numbers, flux decreased during PD and red numbers, increased from the normal condition. Note that for clarity not all separate biochemical steps are plotted: oxaloacetate is for instance first transaminated to aspartate before being
transported across the mitochondrial membrane as part of the malate-aspartate shuttle. GLC, glucose; G3P, glyceraldehyde 3-phosphate; R5U5PD, ribulose-5-phosphate; PYR, pyruvate; LAC, lactate; CIT, citrate; AKG, alpha-ketoglutarate; SUCC, succinate; MAL, malate; OAA, oxaloacetate; GLU, glutamate; GLN, glutamine, GABA, 4-aminobutanoate (synonym of gamma-aminobutyrate); O2, oxygen; OxPhos, oxidative phosphorylation. Flux values from GLC to R5U5PD and from R5U5PD to G3P represent 6-carbon units leaving the GLC pool rather than 3-carbon units entering the G3P pool.

Discussion

Comparison of computational predictions and cerebral metabolic measurements

To test our new method to calculate changes in metabolism from changes in expression of metabolic genes, we compare changes predicted with direct measurements to the extent that these were possible in relatively large brain regions. Measurements of cerebral metabolism in PD by positron emission tomography (PET) have recently been meta-analysed (Borghammer et al. 2010; Borghammer 2012). In 11 out of the 14 studies that were reviewed, 2-32 % decreases in cerebral glucose consumption were reported, although in only four of these cases the change was reported to be significant. In only two of the meta-analysed studies there was a very small (2-4%) and non-significant increase. From the gene expression changes in cortical areas analysed in the present study (GSE8397 and GSE20168; see Supplementary Table S5) we predict a decrease in glucose consumption of about 11%, which is of the same order as the 8.5 % average decrease seen in the meta-analysis of the PET measurements.

In the meta-analysis, the decrease in oxygen consumption in three PD study groups measured by PET ranged from 6-34% (average 19% decrease). From the gene expression changes in the two cortical areas, we computationally predict a decrease of 17.5 and 22% in oxygen consumption. Our predictions for changes in glucose and oxygen consumption for the cortical areas agree with direction and size of change in the PET measurements in PD patients. Our computational predictions are compatible with the conclusion from the meta-analysis of PET measurements that in PD there is cortical hypometabolism (Borghammer et al. 2010; Borghammer 2012).

The spatial resolution of PET measurements is characterized by a Full Width at Half Maximum of at least 5 mm, which in practice is often even considerably larger (Borghammer et al. 2010). In contrast, gene expression measurements were even feasible for laser-excised cells which made computational predictions specifically for dopaminergic neurons possible. Further, in addition to glucose and oxygen uptake, our computational method describes the metabolic pattern in the entire network and therefore has a high ‘biochemical resolution’ while with PET only uptakes of single metabolites are measured. Examples are the computational prediction from the present
study that the relative decrease in ATP synthesis is larger than the decrease in glucose or oxygen uptake, the prediction that the flux in the middle of the TCA cycle is more reduced than at the start and end, limited metabolic rerouting around downregulated enzymes, shifts in redox shuttles and emergence of lactate production, the latter in agreement with NMR measurements (see below).

**Predicted Metabolic Fluxes During Parkinson's disease**

ATP synthesis is driven by protons which are pumped by the ETC complexes from mitochondrial matrix to cytosol and flow back through ATP synthase. In our network model, protons in the mitochondrial matrix are balanced and ATP synthesis by the mitochondrial ATP synthase is determined. Our computational analysis predicts that the proton fluxes through all ETC complexes and ATP synthase in the SNc during PD are reduced by the same proportion (average 18%) relative to the healthy brain. As a result, the predicted uptake of oxygen into the brain and the ratio of oxygen to glucose uptake are reduced.

Our computational analysis predicts that the reduced pyruvate flux into the mitochondria is associated with production of lactate, accounting for about 10% of pyruvate in the GPi region and for about 6% in the substantia nigra and other brain regions. Increase in cerebral lactate in PD has indeed been measured using magnetic resonance spectroscopy in various parts of the brain (Bowen et al. 1995; Henchcliffe et al. 2008).

**Rerouting of pathways**

In the GABA shunt pathway, the flux of alpha-ketoglutarate to succinate in the TCA cycle via alpha-ketoglutarate dehydrogenase (AKGDm) and succinate-CoA ligase (SUOAS1m) is rerouted through decarboxylation of glutamate to GABA via glutamate decarboxylase (GLUDC) in the cytosol, and subsequently to succinate via GABA transaminase (ABTArm) and succinate semialdehyde dehydrogenase (SSALxm) in the mitochondria (Supplementary Figure S1). The GABA shunt is active in GABAergic neurons (Hassel et al. 1998), providing a mechanism for synthesis of GABA which is an inhibitory neurotransmitter. The GABA shunt in general accounts for less than half of the total TCA cycle flux in GABAergic neurons (Balázs et al. 1970; Hassel et al. 1998). GABAergic neurons account for about 18% of total neuronal glucose oxidation (Hyder et al. 2006). The GABA shunt flux is present in glutamatergic and cholinergic neurons, although it is small there (Lewis et al. 2010).

In PD, a marked reduction in alpha-ketoglutarate dehydrogenase (AKGDm) complex by immunostaining has been reported in the substantia nigra of PD patients (Mizuno et al. 1995). Gene expression data associated with AKGDm also show downregulation in PD patients (Supplementary Figure S2). Consistent with this reduced
activity, the computational analysis also predicts lower flux through AKGDm. This reduction can be compensated by rerouting of alpha-ketoglutarate through the GABA shunt. For Alzheimer’s disease (AD), Lewis et al. (Lewis et al. 2010) applied a metabolic model and inferred that the about 50% reduced AKGDm activity measured for AD is compensated by increases in GABA shunt flux in AD. We confirmed this prediction based on the Lsei-FBA analysis of gene expression changes in an AD data set (Gavai et al. 2015). However, in the present study the upregulation of flux in the GABA shunt pathway during PD was predicted to be much smaller than for AD.

**Flux in Globus Pallidus internus is increased**

The present flux analysis predicted total cellular ATP production in the GPi region to be higher during PD, accompanied by increased fluxes in all pathways (Figure 2D). This may be associated with the role of GPi in the neural circuits that regulate human movement. In PD, loss of dopaminergic neurons in the striatum causes hyperactivation of the subthalamic nucleus (STN) and GPi, leading to increased neuronal firing rates in the GPi (Hutchison et al. 1994) and disturbed regulation of motor neurons (Dostrovsky et al. 2002; Baunez & Gubellini 2010). This theory has been the basis of deep brain stimulation (DBS) treatment in PD patients targeting the GPi and STN region (Dostrovsky et al. 2002). There is therefore a striking correspondence between the increase in metabolic fluxes in the GPi and increased neuronal activity in this region. A remarkable detail is that in spite of increased oxygen uptake, lactate efflux in the GPi is predicted to be increased.

**Limitations and prospects of the study**

By computational analysis we predicted changes in metabolic fluxes in small regions in the brain, such as the substantia nigra. In relatively large cortical regions the metabolic rates for glucose and oxygen were measured with PET and agree with our computational predictions. Metabolic changes in small regions such as the substantia nigra, and in particular in dopaminergic neurons in this region, could not a priori be assumed to be the same as changes determined in larger regions which are accessible to experimental flux measurements with low spatial resolution. However, the present computational analysis predicts changes in the SN that are similar to other brain regions. Also the results for laser-captured dopaminergic neurons are similar to the whole SN and most other brain regions. In contrast, one particular brain region, the GPi, shows different metabolic changes than the other brain regions, including the SN, which usually is most prominently affected by PD. Our computational prediction therefore suggests that during PD, metabolism is decreased similarly in most brain regions. However, the GPi represents a small region where metabolism is increased in parallel with increased neuronal activity.
The predicted changes in metabolism are averages for the region sampled based on gene expression changes measured for the sample as a whole. There are several distinct cell types inside these regions. The disease may have progressed much more in some of the cells than in others, and damage may even be heterogeneous in cells of the same type. The changes in metabolic fluxes may therefore be larger in a subset of the cells than in the tissue as a whole. Because neurons and glia are lumped in the mRNA expression measurements, we also used a model which lumps metabolism of neurons and glia. Models of brain metabolism with separate compartments for neurons and glial cells exist (Cakir et al. 2007; Lewis et al. 2010), but have no added value in this case because the available gene expression measurements reflect a weighted average of cell types. For the present analysis a lumped model was therefore used with biochemical reactions not compartmentalized in distinct cell types. The use of a metabolic model with one compartment for tissues which contain several cell types means that limitation of metabolism by exchange processes between the cells is assumed to be negligible. The correspondence between metabolic rate measurements and computational predictions for cortical regions, see above, suggests that this assumption is reasonable.

Among the SN expression datasets included in the study by Zheng et al. (Zheng et al. 2010), the dataset from Cantuti-Castelvetri et al. (Cantuti-Castelvetri et al. 2007) (GEO accession GSE24378) differs from the rest by displaying overexpression during Parkinson’s disease in most of the genes in the metabolic pathways. As suggested by (Zheng et al. 2010), this may be caused by the use of the non-standard X3P microarray chip, which differs from the rest of the platforms used. For this reason this data set was not included in the final analysis of the present study. Our analysis on the GSE24378 data set indeed predicted that most metabolic fluxes are upregulated (see Supplementary Table S4), which differs from the results for all other SN data sets.

The present prediction is based on gene expression changes. Regulation of translation of mRNAs in proteins and breakdown and posttranslational modification and allosteric regulation of enzymes in the metabolic network may modify the relation between mRNA expression and flux. The relation between changes in gene expression and metabolic fluxes was investigated for glycolysis in yeast (Daran-Lapujade et al. 2007). Only a fraction of the enzymes involved in yeast glycolysis showed clear changes in gene expression in the same direction as the change in flux carried by that particular enzyme. A more recent approach presents flux prediction based on absolute gene expression data on a large scale yeast network (Lee et al. 2012). The latter method is able to meaningfully predict flux compared to exo-metabolome measurements. In our study, the changes in gene expression in metabolic pathways in PD (Supplementary Figure S2) appeared to be more consistent and uniform than in the study on yeast glycolysis. This may explain why the computational predictions based on gene expression changes in the present study agree with the changes in metabolic rate measured by PET (see above).
The approach by Lee et al. (2012) in yeast and our present approach have a common assumption that metabolic fluxes tend to be related to gene expression without assuming a rigid relation for each individual reaction. Both studies suggest that it is useful to take the metabolic network connectivity into account to estimate an overall effect of gene expression on the metabolic flux.

Several other algorithms exist to predict metabolic fluxes from gene expression data. These algorithms, such as iMAT (Shlomi et al. 2008), GIMME (Becker & Palsson 2008), GX-FBA (Navid & Almaas 2012), E-Flux (Colijn et al. 2009), Lee-12 (Lee et al. 2012), RELATCH (Kim & Reed 2012) have recently been extensively reviewed and benchmarked on yeast and E. coli data (Machado & Herrgård 2014). In the original publication on Lsei-FBA, the algorithms tested by Machado were tested on gene expression data for brain tissue (Gavai et al. 2015) and appeared to perform better for this application than the algorithms benchmarked by Machado et al. (2014). The characteristics of Lsei-FBA in comparison with these other algorithms have been extensively discussed by Gavai et al. (2015).

Our approach has a limitation which is specific to brain tissue: a fraction of the enzymes which are formed from the measured messenger RNAs are transported over relatively long distances to catalyse metabolism in axonal terminals. Many dopaminergic neurons in the SNc receive for instance input via GABAergic synapses from relatively distant GABAergic neuronal cell bodies (Bolam and Smith 1990). The predicted metabolic changes therefore apply to the cells whose gene expression levels are measured, which includes distant nerve terminals of those cells, but excludes metabolic changes in nerve terminals from distant neuronal cell bodies that extend into the region where mRNAs are sampled. This means that metabolic changes predicted from gene expression changes on the one hand, and directly measured in the same region on the other hand, may diverge to a certain extent.

**Conclusion**

This paper describes application of a recent method to predict changes in metabolic fluxes based on changes in gene expression in patient material. From gene expression changes during Parkinson’s disease, metabolic fluxes through central carbon metabolism are predicted to be reduced in the substantia nigra and other brain regions including frontal cortex, cerebellum and putamen. A striking result is that the predicted relative changes in ATP synthesis are larger than the changes in glucose uptake. We also predicted increase of lactate production and shifts in redox shuttles. Reduced metabolism via alpha ketoglutarate dehydrogenase in the middle of the TCA cycle is less compensated via the GABA shunt than is the case in Alzheimer’s disease. In contrast to the decreases in metabolism in substantia nigra and most other brain regions, the
globus pallidus internus part of the brain is predicted to show increased metabolic flux compared to normal controls.

Competing interest

The authors declared that they have no competing interest

Author contributions

JHGMvB initiated and supervised the project; FS designed the model and conducted the model simulation and analysis; FS and JHGMvB wrote the manuscript.

Supplementary materials

Supplementary Table S1 – List of reactions in the model
Supplementary Table S2 – List of metabolites in the model
Supplementary Table S3 – Summary of all datasets used in this study
Supplementary Table S4 – Results for flux prediction for substantia nigra and dopaminergic neuron datasets
Supplementary Table S5 – Results for flux prediction for substantia other tissues
Supplementary Figure S1 – Reconstructed metabolic reaction network
Supplementary Figure S2 – Visualisation of fold changes in mRNA expressions mapped on the metabolic pathway model

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