Investigation of Serotonergic Parkinson's Disease Related Pattern and Altered Dopamine Release Pattern in Treatment-Induced Complications and Non-Motor Symptoms of Parkinson's Disease

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Abstract

Parkinson's Disease is the second most common neurodegenerative disorder. Apart from motor symptoms, cognitive deficits are also common. Treatments, mainly in the form of dopamine (DA) replacement therapy, although reduce motor symptoms at first, can lead to treatment-induced complications. Abnormal spatial covariance metabolic pattern linked to the motor and cognitive symptoms of Parkinson's Disease (PD) have previously been defined using Fludeoxyglucose Positron Emission Tomography (PET). In contrast, little is known about the functional networks in the serotonergic system, which is known to be closely related to cognitive dysfunctions of the disease.

In this thesis work, we want to investigate the interactions between the dopaminergic and serotonergic pathways in presymptomatic and early stages of the disease, and their contributions to treatment-induced complications and non-motor symptoms in PD subjects.

In the first part of this project, we investigated the PD and LRRK2 mutation related patterns in the serotonergic system by studying 12 asymptomatic LRRK2 mutation carriers (LRRK2-AMC), 9 healthy controls (HC), and 18 PD subjects using [¹¹C]-3-amino-4-(2-dimethylaminomethylphenylsulfanyl)-benzonitrile (DASB) PET and a principal component analysis (PCA) based regional covariance model with bootstrap resampling. The serotonergic PD-related pattern (SPDRP) significantly separated PD subjects from HC subjects (p< 0.0001). A distinct asymptomatic LRRK2 mutation-related pattern (LRRK2-AMRP) significantly separated LRRK2-AMC with HC subjects (p< 0.0001).

In the second part of the project, we analyzed the medication-induced DA release pattern for 10 early PD subjects using double $[^{11}C]$ -Raclopride scans. We found a significant negative correlation between DA release and age of onset in the striatum.

These findings, although obtained with a small number of subjects, suggest that the serotonergic system may be affected by PD in a specific pattern and regions relatively preserved binding may contribute to cognitive dysfunctions related to PD. LRRK2-AMC subjects showed a distinct pattern, which indicates that either such increase is of compensatory nature or is a characteristic of this specific mutation. The combination of abnormal medication-induced DA release pattern and upregulation of the serotonergic system may be able to explain the occurrence of treatment-induced complications and non-motor symptoms in PD patients, and act as a potential early marker for the disease.

Preface

Part of this thesis work (Chapter 4) was presented at 11th International Symposium on Functional NeuroReceptor Mapping of the Living Brain. Full reference can be found at J.Fu, N.Vafai, E.Shahinfard, N.Heffernan, J.McKenzie, R.Mabrouk, I.Klyuzhin, A.J.Stoessl, V.Sossi, 2016, Investigation of Parkinsons Disease Related Covariance Pattern in the Serotonergic System using [11C]-DASB/PET, 11th International Symposium on Functional NeuroReceptor Mapping of the Living Brain, Boston, USA, July 13-16.

This study was approved by UBC Research Human Ethics Board, in particular the Clinical Research Ethics Board, under 'The Evolution of PD' (certificate number: H12-00843).

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Chapter 1

Introduction

1.1 Positron Emission Tomography

Positron Emission Tomography (PET) has been widely used to provide information about numerous neural pathways and abnormal patterns of neural activity in patients with neurodegenerative diseases. In this section, I will briefly discuss basic principles behind PET radiotracers, radioisotope decays, signal detection and image reconstruction.

Overview

Positron emission tomography (PET) is a nuclear imaging technique which uses radiotracers to construct a 3D image based on the spatial and temporal distribution of the tracers and provides functional information of the tissues of interest *in-vivo*. PET enables the monitoring of molecular or cellular processes for varies diagnostic or therapeutic applications. Since patients with brain disorders often show distinct metabolic patterns under PET scan, PET imaging offers the possibility to determine *in-vivo* multiple aspects of physiological processes for the study of varies neurodegenerative diseases.

The radiotracer tagged with a radioisotope is introduced into the patients body. The radioisotope decays to produce positrons. Annihilation between a positron and an electron produces two 511 keV gamma rays flying off in opposite directions along a random orientated line. The two gamma rays are detected in coincidence by a pair of scintillation detector elements. The imaginary line that joins the location where the two gamma rays are detected is used to assign those matching gamma rays to a specific line of response (LOR). The measured counts in each LOR are used to produce a 3D image of the object being studied via various image reconstruction algorithms. Details of the process will be discussed the following sections.

Table1.1 summarizes characteristics of common imaging modalities. Compared to other imaging modalities, PET has the highest sensitivity and specificity, but rel-

	Spatial Resolution	Temporal Resolution	Sensitivity (mol l^{-1})
PET	4-6 mm	5-10 s	$10^{-11} - 10^{-12}$
SPECT y	8-11 mm	5-10 s	$10^{-10} - 10^{-11}$
MRI	0.05 - 1.5 mm	0.1-5 s	$10^{-3} - 10^{-5}$
CT	0.05-0.8 mm	0.1-0.5 s	not well defined
Ultrasound	$0.05\text{-}0.5~\mathrm{mm}$	0.005-1 s	single microbubbles

Table 1.1: Comparison between commonly used imaging modalities. [1]

atively low temporal and spatial resolution. On the other hand, magnetic resonance (MR) imaging and computed tomography (CT) can provide high spatial resolution and high soft tissue contrast. Hybrid imaging such as PET/CT and PET/MR have been adopted to combine anatomical and metabolic information. In the field of brain research, PET/MRI provides more comprehensive investigation of brain organization and physiology by looking at metabolic and functional information at the same time. For example, in the study of brain connectivity, structural or functional connectivity from MRI can be combined with metabolic connectivity from PET to provide more specific, sensitive and quantitative measurements, and therefore yield new insights into the brain [12][1].

1.1.1 Radiotracer

A radiotracer is a biological molecule (tracer) tagged with a radioisotope. The choice of tracer and molecule depends on the tissue to be studied and questions of interest.

In oncology, fluorodexoyglucose (FDG), which is a glucose analog that is taken up by glucose-using cells, is often used as the tracer. FDG molecules are tagged with radioisotope fluorine-18 (F-18), which has a half-life of 110 mins. These radioactive $[^{18}F]$ -FDG molecules are trapped in cells until decay. Since cancerous cells take up more glucose than normal cells, FDG/PET can be used for diagnosis, staging and monitoring treatment of various cancers. In neuroimaging, regions with higher brain activity have higher radioactivity. Since brain has a high uptake of glucose and many neurodegenerative diseases, such as Parkinson's disease and Alzheimer's disease, decrease the brain metabolism in certain brain regions, FDG/PET can be also used for neuroimaging [13]. Other tracers, such as raclopride, dihydrotetrabenazine and fluorodopa, are used to target specific physiological pathways related to various diseases. Details about a few radiotracers relevant to this project will be discussed in later sections. Once inside the body, the radio-labeled molecule can 'tracer' out its path. Ideally a tracer should have the following properties:

- 1. specific to the process or site of interest, which can be difficult since tracer in bloodstream can be carried away from the site of interest
- 2. no metabolism after injection. Metabolites can carry labeled isotopes away from the site of interest and cause problems in data interpretation
- 3. easily synthesized from available precursor. The synthesis process should take a relatively short-time before radioisotope activity decays, and have a good yield

Isotope	Half-Life (mins)	Maximum Energy (MeV)	Range in Water (mm)
^{18}F	109.7	0.635	1.03
^{11}C	20.4	0.96	1.86
^{13}N	9.96	1.19	2.53
$^{15}0$	2.07	1.72	4.14

Table 1.2: Characteristics of commonly used PET radioisotopes. The range in water is the total distance traveled by the positron before it annihilates with an electron. [2]

PET imaging depends on positron-emitting isotopes. Radioisotopes can be produced by a cyclotron, or as bi-products of a nuclear reactor, or in a generator system. Short-lived isotopes, such as ${}^{11}C$ and ${}^{15}0$, have to be produced by an in-house or nearby cyclotron. Some commonly used isotopes are listed in Table1.1.1. Isotopes suitable for PET imaging have the following characteristics:

- 1. half-lives are long enough for the duration of the scan, but not too long to avoid unnecessary patient exposure to radiation
- 2. the decayed positrons have relatively low energy and short range before annihilating with electrons in the body to avoid inherent error in the data
- 3. targets are easily available for isotope production
- 4. do not change the biochemical properties of the labeled tracer molecules

The labeled radiotracers have the same physiological properties and hence same tracer kinetics as the unlabeled biological molecules. When the radioisotope decays by emitting gamma rays, distribution of the radiotracer is mapped as a function of time and space by the detected gamma rays. The spatial and temporal distribution of the tracer provides functional or metabolic information of the tissue depends on its biochemical or metabolic properties without disturbing the normal tissue function. The low amount of radiotracer administered to the patient does not induce any pharmacological effect nor affect the biological process under observation [14].

1.1.2 Radioisotope Decay

Radioactive decay is based on the unstable nucleus with too many neutrons or protons which disrupts the balance between attractive and repulsive forces in the nucleus. the Coulomb force (repulsive) and the strong force (attractive). Unlike stable nuclei, unstable nuclei do not have enough attractive force to hold the nuclei permanently together, and are therefore radioactive.

Positron emission is a particular type of radioactive decay, in which a proton in a proton-rich nucleus (X) is converted to a neutron while releasing a positron (β^+) and a neutrino (v):

$${}^{A}_{Z}X = {}^{A}_{Z-1}Y + e^{+} + v \tag{1.1}$$

After converting proton to neutron, the nucleus decays to its stable form (Y). Positron is emitted to conserve electric charge.

Annihilation occurs when a subatomic particle collides with its antiparticle, in this case, when a positron collides with an electron. Due to energy and momentum conservation, low-energy annihilated particles are replaced with two gamma ray photons. Since both electron and positron have a rest energy of 511k electron volts (eV), this energy is given off equally to two gamma rays. Energy and momentum are conserved with 1.022 MeV of gamma rays traveling at opposite directions, which are detected by PET detection system [4].

Emitted positron undergoes many scattering events along its path in the medium before annihilates with an electron, so the actual path length the positron travels before annihilation (p) is greater than the positron range (r) as shown in Figure 1.1. Corrections are made to account for the degrading effects of positron range, especially for isotopes with high positron range. Many have used Monte Carlo simulations or high resolution optical methods to model the positron range distribution. [2]



Figure 1.1: Nucleus decays to emit positron (β^+) which travels to the site of annihilation where it annihilates with an electron (e^-) producing two 511 keV gamma rays (γ) in opposite directions. r is the displacement from the parent nucleus to the site of annihilation, whereas p is the actual path of β^+ [4]

Radioactive Decay Rates

For a given radioactive nucleus, the exponential probability of decay is described using decay constant λ , the probability that a nucleus will decay per unit time. The radioactivity (A) of a sample is the number of decays per unit time in the units of Becquerel (decays/second). N is the number of radioactive atom in the sample. The half-life ($T_{1/2}$) is the time when activity of sample has halved.

$$N(t) = N_0 e^{-\lambda t}$$

$$A(t) = \frac{-dN(t)}{dt} = \lambda N(t)$$

$$T_{1/2} = \frac{ln(2)}{\lambda}$$
(1.2)

1.1.3 Detection System

Detection system is a key component to obtain quantitative information from the imaging system. PET scanner detects the two gamma rays originating from positron annihilation using scintillation detectors. The annihilation photons (511 keV) traveling in opposite directions form a line of response (LOR) are detected in coincidence (i.e. by searching for light signal at this energy within a very short time window within a few nanoseconds) by a pair of detector elements surrounding the part of the body being scanned (in our case, the brain).



Figure 1.2: A schematic of PET block detectors which is based on the first commercial human PET scanner built in 1974. The scanner was made by 48 NaI(T1) detectors. [4][5]

Scintillation

Scintillating crystals detect the gamma rays and convert them to scintillation light via the following steps [5]:

- 1. Incident photon on the scintillating crystal creates an energetic electron by Compton scatter or photoelectric effect
- 2. Electron loses its energy as it passes through the scintillator, and excites other electrons along the way
- 3. Excited electrons return to their ground state, releasing energy in the form of visible light

Some photons may scatter off the detector and only deposit portion of their energy in the scintillator, especially in small detectors, but increasing detector size reduces the spatial resolution of the system. So choosing the ideal scintillator material based on the following criterion is important to have optimal scintillator performance:

- high effective atomic number (Z). High Z materials have high linear attenuation coefficients, which increase the proportion of photons undergoing photoelectric absorption, thus increasing sensitivity of the scintillator
- high light yield, meaning that incident photons should produce a large number of scintillation photons
- low self-absorption for the scintillation light
- index of refraction close to glass, which improves the optical coupling between the scintillator and photomultiplier tubes

Photomultiplier Tubes

The scintillator is coupled with the photomultiplier tubes (PMTs) (as shown in Figure1.2), which then generate electric signal in response to the light incidence and send the signal to computers. The scintillating photons incident on the surface of PMTs result in a short electrical pulse, which is then further amplified by electronics and coincidence circuitry. When two signals from opposing detectors arrive in coincidence along a line of response (LOR) and sends this information to a computer. In contrast to single photon emission computed tomography (SPECT), PET does not need physical collimator to determine the direction of the incident photons [6].



Figure 1.3: a) two annihilation: X is detected by detector elements D3 and D67 along line of response (LOR), Y is undetected because photon path does not interact with detector ring. b) top view of annihilation of X [6]



Figure 1.4: True coincidence detection vs scatter and random coincidence detection in PET [7]

Photon Interaction and Attenuation Correction

When the two photon beams travel through the medium, they can interact with human tissues via Compton scatter or photoelectric absorption.

In Compton scattering, which is the most dominant interaction for 511 keV photons, a photon interacts with an electron, which results in decrease in photon

energy (increase in wavelength) and change in the direction of the photon. This lost energy is transferred to the recoil electron, and the energy of the scattered photon after interaction is given by [15]:

$$E' = \frac{E}{1 + (E/m_0 c^2)(1 - \cos \theta)}$$
(1.3)

where E' is the energy of the scattered photon, E is the energy of incident photon, m_0c^2 is the rest mass of an electron, and θ is the scattering angle.

In photoelectric absorption, the incident photon is completely absorbed and an energetic electron is ejected from the outer bound shell of the atom [15]. In human tissues, the probability of photoelectric absorption is low for photon with 511 keV energy [16].

Compton scattering and other interactions lead to attenuation of the two 511 keV photons. The number of photons pass through an attenuating material decreases exponentially with increasing length of the material. For 511 keV photons, about 7cm thickness of tissue is needed to reduce the number of photons to half.

The probability that a photon will reach the detector is given by:

$$P = \exp^{-\int_0^x \mu(x)dx} \tag{1.4}$$

where P is the probability a photon will reach the detector at distance x through some attenuating material, and μ is the linear attenuation coefficient.

Because the interaction probabilities for the two photons are independent of each other, the total probability that both photons will reach the detector and been recorded as a coincidence event is given by [16]:

$$P_c = \exp^{-\int_0^L \mu(x)dx} \tag{1.5}$$

where L is the distance between two detectors.

Coincidences Detection and Correction

As a result of annihilation, we expect the two photons to arrive at the detectors at approximately the same time. Temporal mismatches (photon detection not occurring at the same time) may occur due to the finite timing resolution of the scintillation crystal and the processing time of the PMT. These timing uncertainties are taken into account using the coincidence time-window, usually in the order of 6-10ns [17]. When annihilation occurs at a location closer to one detector than the other, there will be a slight delay from one photon than the other. This can be corrected with time-of-flight PET imaging, which uses the relative time difference (Δt) between detection of two photons to estimate the most likely location of the annihilation event along the LOR.

There are 4 different kinds of coincidence events in PET: true, scatter and random (as shown in Figure 1.4).

As mentioned before, true coincidence event occurs when two 511 keV photons from annihilation are detected by scintillators at the same time. Photons do not undergo any interaction before detection and no other event is detected within this coincidence time-window [17].

Random coincidences occur when two detected photons are actually originated fro two separate annihilation events, which can be corrected using a delayed coincidence circuit. Scattered coincidences are caused by scattered photons within patient body as a result of Compton scattering. Even though two photons are originated from the same annihilation event, since the direction of the photon is changed, the LOR does not cover the true location of this event. This incorrect LOR assignment can be corrected using complex simulation methods [7]. These scattered and random coincidences add noise to the signal and decrease image contrast.

Attenuation Correction Attenuation due to interaction between photons and tissues can be corrected using attenuation correction (AC). Because tissues with different densities have different attenuation abilities, less dense regions (e.g. lungs) will appear darker (more photon emissions) than more dense regions (e.g. bones) without AC, which can lead to inaccurate estimation of tracer uptake. To perform AC, we need to obtain the attenuation map from all LORs. On stand-alone PET scanners, a transmission scan is usually performed, in which an external positron source is rotated around the patient to determine the attenuation of this transmission photon beam [18]. In PET/CT scanners, CT images can be used for PET AC [19].

The collected data are the counts for the number of coincidences $[n^*(l), \ldots, n^*(D)]$ where $n^*(d)$ is the total number of coincidences counted by the dth detector pair and D is the total number of detector pairs. Note that not all photons reach the detector due to attenuation inside the body and detector. Considering different factors affecting the counts of coincidence events, the following equation is often used to estimate loss of counts (or attenuation)

$$Y_{d\theta} = \gamma_{d\theta} [\eta^t_{d\theta} P_{d\theta} M_{d\theta} + \eta^r_{d\theta} r_{d\theta} + \eta^s_{d\theta} s_{d\theta}]$$
$$P d\theta = \exp^{-\int \mu(x) dx}$$

where $\mu(x)$ is the linear attenuation coefficient at position x, M is the number of annihilation along LOR specified by (d, θ) , P is the survival probability (probability of a photon not interacting along LOR), r is the number of accidental coincidences, s is the number of scattered events, η is the probability of each corresponding event, γ is the probability of event not being lost due to deadtime [20][21].

1.1.4 Image Reconstruction

After corrections for attenuation, scatter and random effects, the number of counts along each LOR is proportional to the line integral of the activity along that LOR, which is known as projection. Radiotracer distribution inside the body is modeled using 3D volume elements (voxels), and f is the true image which can be represented as the number of β^+ decays at each spatial location corresponding to each voxel. If the number of coincidence events along each LOR is vector p, the system matrix H relates radioactive decay inside the body to coincidence events recorded by the detector. The imaging system is described as:

$$p = Hf \tag{1.6}$$

Given p and H, we can estimate f through varies image reconstruction algorithms. The reconstruction algorithm of choice depends on the question of interest, and there is always a trade-off between the signal-to-noise ratio (SNR), contrast, bias and resolution.

There are two common types of PET image reconstruction algorithms, the iterative and analytic algorithms. Iterative algorithms are very flexible and require no constraints on the system model, but can be very computationally expensive. Analytic algorithms, on the other hand, are much faster, but have higher noise and limited quantification accuracy [4][22].

Analytical Reconstruction

Analytic reconstruction, such as the filtered back projection (FBP) algorithm, requires the system matrix H to be simplified. In the analytic approach, a finite number of projections is applied back to the image to obtain a rough estimation of the true radiotracer distribution. Star-like artifacts resulting from the limited number of projections can be improved using a ramp filter. The combination of the back projection and the ramp filter is the FBP method. In the analytic approach, no statistical model is included, which lowers the SNR [23].

Iterative Reconstruction

Compared to analytic reconstruction algorithms, iterative reconstruction approximates the real solution of the object using multiple iterative steps, which allows us to reconstruct better images but at a higher cost of computational time. Iterative reconstruction algorithm has the advantage of improved noise insensitivity, which is particular interest for images with poor noise statistics like PET [23][22].

In iterative reconstruction approach, we need to define the parameters to estimate (represent radiotracer concentration) and the system model which relates the radiotracer distribution and the mean of the measured data.

The system matrix Hij, which is the probability that an emission from voxel j is detected in projection i, characterizes the imaging system. The projection p_i is given by [24]:

$$p_{i} = \sum_{j=1}^{N} H_{ij} f_{j}$$
(1.7)

After acquiring the projection measurements, a statistical model is used to describe how the projection measurements vary around the expected mean. A cost function is often used to define the 'best' image, and the Maximum Likelihood approach is most commonly used for PET since it offers unbiased, minimum variance estimates as the number of measurement increases.

1.2 Kinetic Modeling

To obtain quantitative measurements from physiological tracer distribution, we often need a kinetic model to relate PET data to tissue functions. In these models, radiotracer moves between different tissue compartments, which represent different biochemical states of the radiotracer and its metabolites. By assumption, there is an uniform radiotracer distribution inside each compartment.

In a compartment model, there a fixed number of states with specific interactions among them and arrows represent pathways where radiotracers flow between each compartment. The change in concentration in each tissue compartment is described by a linear, first-order ordinary differential equations (ODE) of the concentrations in all other compartments. From these ODEs, tracer kinetics are the convolution of the input function and response function in other compartments.

Compartment models can be used to fit the tissue concentrations as a function of time from the measured PET data. Kinetic models used in PET quantification are discussed in the following sections.

1.2.1 Time Activity Curves

The time activity curve (TAC) gives the radioactivity value in each region-of-interest (ROI) or pixel across a sequence of PET images (i.e. scanning time) as shown in Figure 1.5. Radioactivity of the tracer reaches maximum then stabilizes as the isotope decays. To quantify this curve, we need to fit compartment models to obtain quantitative information.

Standard Uptake Values

Standard uptake values (SUV) can be estimated from TACs. SUV is defined as the ratio of 1) the mean tissue radioactivity concentration c of ROI (Mbq/kg), and 2) the injected activity (Mbq) divided by the body weight (kg). SUV(t)=c(t)/(injected activity(t) / body weight). SUV represents the ratio of (1) the image derived radioactivity concentration found in certain ROI, and (2) as reference the radioactivity concentration in the hypothetical case of an even distribution of the injected radioactivity across the whole body. Average SUV in each ROI was also obtained over the last 30mins (50-80mins) time frame. SUV can be significantly affected by image noise, low image resolution and/or user biased ROI selection.

1.2.2 Reference Tissue Model

The commonly used reference tissue model (RTM) in PET neuroscience studies is illustrated in Figure 1.6 and parameters are explained in Table 1.2.2. The reference region is modeled as a 'single-tissue' compartment (C_R) , while the target region is modeled as a 'two-tissue' compartment $(C_{ND}andC_S)$.

 C_P is the arterial input function, which represents the cumulative availability of the radiotracer in arterial plasma. Tissue concentration normalized to the cumulative arterial concentration is often used as the gold standard for quantitative PET



Figure 1.5: Time activity curve for [11C]-DASB tracer with SRTM model fit in the left caudate region. The estimated parameters from SRTM are shown. Error bars are estimated from the scanner count-rates [4]

studies. However, since getting arterial blood samples during the scan can be a challenge sometimes, arterial input function can be sometimes replaced by reference tissue if one exists.

The rate of which tracer moves from one compartment to another is proportional to the tracer concentration in the first compartment [3]. For the non-displaceable compartment C_{ND} , we have the following differential equation describing the change in radioactivity concentration:

$$\frac{dC_{ND}}{dt} = K_1 C_P - k_2 C_{ND} - k_3 C_{ND} + k_4 C_S \tag{1.8}$$

In RTM, the two target tissue compartments do not represent different physical spaces. The specifically bound compartment C_S is where tracers bind to their specific target. The non-specifically bound compartment C_{ND} is where tracers may



Figure 1.6: Systematic diagram of the reference tissue model [8]. Using a reference region, parameter Cp can be eliminated.

either bind to non-target molecules or unbound (free).

The reference tissue compartment C_R , which is assumed to have no specific binding but similar non-specific binding as the target compartment, is used to estimate the input function without measuring tracer concentration in plasma C_P . The reference tissue compartment only contains non-specifically bound and free tracer [3][8].

For RTM, the operational equation is given by:

$$C_T(t) = R_1[C_{R(t)} + a \cdot C_R(t) \bigotimes e^{-ct} + b \cdot C_R(t) \bigotimes e^{-dt}]$$
(1.9)

where t is the time after tracer administration and \bigotimes is convolution.

 R_1 is defined as the ratio of tracer deliver rates between the target and reference regions:

$$R_1 = \frac{K_1}{K_1'} \tag{1.10}$$

Symbol	Definition	Units
C_P	Tracer concentration in plasma	$kBq.mL^{-1}$
C_{ND}	Tracer concentration in non-specifically bound	$kBq.cm^{-3}$
	target tissue	
C_S	Tracer concentration in specifically bound target	$kBq.cm^{-3}$
	tissue	
C_R	Tracer concentration in reference tissue	$kBq.cm^{-3}$
K_1	Rate constant for transporting tracer from arte-	$mL.cm^{-3}.min^{-1}$
	rial plasma to reference tissue	
K_1'	Rate constant for transporting tracer from arte-	$mL.cm^{-3}.min^{-1}$
	rial plasma to reference tissue	
k_2	Rate constant for transporting tracer from ref-	min^{-1}
	erence tissue to venous plasma	
k'_2	Rate constant for transporting tracer from ref-	min^{-1}
	erence tissue to venous plasma	
k_3	Rate constant for transporting tracer from non-	$ min^{-1}$
	displaceable to specifically bound compartment	
k_4	Rate constant for transporting tracer from	min^{-1}
	specifically bound to non-displaceable compart-	
	ment	

Table 1.3: Symbols used in the reference tissue model, as shown in Figure 1.6. [3][4]

The parameters a,b,c,d can be estimated from different combinations of rate constants. Because we assume that the volumes of distribution for non-displaceable tracer are equal in the reference and target region, so that $\frac{K1}{k2} = \frac{K1'}{k2'}$. This assumption allows us to reduce the number of independent parameters from five (a,b,c,c and R1) to four (R1,k2,k3,and BP_{ND}), which are then estimated using nonlinear regression analysis [8]. The two assumptions of RTM are: 1) there is no specific binding in the reference tissue compartment and 2) K1/k2 is the same in reference and target tissue compartments.

Binding Potential

The goal of PET study is to estimate all the rate constants in the compartment model using the fit parameters. Instead of estimating these rate constants directly, which is prone to statistical noise in model fitting, more robust parameter is used to combine these rate constants. Because the rate constants are highly covariated, the overall error for the combined parameter is less than the error for each rate constant.

In a ligand receptor binding system, ligand-receptor kinetics is described by the Michaelis-Menten equation.

$$L+R\leftrightarrow LR$$

where L = ligand, R = receptor, LR = ligand-receptor complex.

Binding potential (BP) is most commonly used to estimate the rate constants. In PET, BP values are combined measures of availability and affinity of neuroreceptors, and is the ratio of Bmax oto K_D :

$$BP = Bmax/K_D = receptor density x affinity$$
(1.11)

where Bmax is the total concentration of receptors in the tissue, and K_D is the radioligand equilibrium dissociation constant.

There are different definitions of BP, but for our interest, non-displaceable binding potential (BP_{ND}) is used, which defined as [3]:

$$BP_{ND} = \frac{k_3}{k_4} \tag{1.12}$$

1.2.3 Simplified Reference Tissue Model

RTM has four parameters to be estimated which is often too complex for the noisy PET data. In most cases, it can be replaced by the simplified reference tissue model (SRTM).

Compared to the original RTM, SRTM reduces the number of tissue compartments to one instead of two (i.e. combined the specifically bound compartment C_S and non-specifically bound compartment C_{ND}). This reduces the number of parameters from four to three (eliminate k3) and reduces the variability in the parameter estimates. SRTM is used to quantify the receptor kinetics from PET measurements using input function derived from a reference region without acquiring an arterial input function [8] [25]. The three parameters used in SRTM are R1 (relative delivery in tissue compartment compared to the reference region), k2' (the clearance rate constant from the reference region) and BP_{ND} (k3/k4) estimated using nonlinear fitting or more complex models.

SRTM uses the following three assumptions to estimate specific binding in tissue regions of interest as a function of the reference region [25][26]:

- 1. reference tissue compartment has no specific binding
- 2. the volumes of distribution for non-displaceable tracer are equal in the reference and target region, so that $\frac{K1}{k2} = \frac{K1'}{k2'}$.
- 3. there is no difference between the specific and the non-specific compartment, so TAC can be fitted by an one-tissue compartment model.

SRTM was used to generate BP_{ND} values for covariance pattern analysis in the serotonergic system, which was the main part of this thesis work.

1.2.4 Two-Step Simplified Reference Tissue Model

SRTM calculates one BP_{ND} value for each ROI using regional TAC, but sometimes parametric images of BP_{ND} values are of particular interest. In parametric images, each voxel is related to some physiological parameter. This is done by applying traditional model to each individual voxel separately.

Although there is only one true value of k2', SRTM estimates k2' value for each pixel of the image. A two-step method (SRTM2) was developed [25][26]:

- 1. R1, k2 and k2' values are calculated using SRTM for all brain pixels. A global k2' value is calculated from all pixels outside the reference region.
- 2. Fix k2' value to the averaged global value and calculate functional images of BP and R1 using a two-parameter fit.

SRTM2 was used to generate parametric BP_{ND} images for DASB tracer and was compared with regional BP_{ND} values from SRTM.

1.2.5 Logan Plot Method

Compared to RTM or SRTM, data fitting using linear regression methods is in particular interest due to faster computational time . Logan plot is a graphical method which reduces the number of parameters by transforming the model equation (1.9) to a linear equation evaluated at several time points and interpret the slopes and intercepts of the linear equations [27]. This method is independent of the specific model structure of the reference tissue and uses a global clearance rate k2' as SRTM2. Logan plot method was used to generate regional BP_{ND} values for RAC and DTBZ tracers.

1.3 Network Analysis

Functional imaging techniques allow us to quantify brain activity to study pathophysiology of neurodegenerative disorders, but absolute activity may not provide the complete picture. In addition, activity variability between subjects and brain regions increases the difficulty to quantify PET signal.

Moeller and colleagues [28] proposed a data-driven, statistical regional covariance model based on multivariate principal component analysis (PCA), namely the Scaled Subprofile Model (SSM). SSM models the sources of subject and region variation as spatially distributed networks in functional images. SSM is able to identify a group-dependent, region-specific, disease specific spatial covariance patterns in the brain that can be used study the heterogeneous regional interactions in different patient groups and to discriminate patients from healthy controls [29][30]. Network analysis has been proven to be more robust than local binding analysis and more sensitive to small changes. For the serotonergic system, which is the main focus of this thesis work, most studies on serotonergic pathways have been focused on local binding, disease-specific alteration of the functional network across the entire brain is still unknown.

1.3.1 Overview

Disease-specific metabolic network abnormalities have been used to accurately discriminate between PD patients and controls using SSM. The so-called PD-related pattern (PDRP) derived from ¹⁸F-fluorodeoxyglucose (FDG) PET was characterized by increased pallido-thalamic and pontine activity associated with relative reduced activity in the cortical motor regions [31] [32] and was found to correlate consistently with Unified Parkinson's Disease Rating Scale (UPDRS) motor scores [30] and clinical response to therapy [33].

Similar network analysis has also been applied to identify the PD-related cognitive pattern (PDCP) using FDG as a potential biomarker of cognitive functioning in PD. PDCP pattern was characterized by relative increased activity in the cerebellar vermis and dentate nuclei with associated reduced activity in frontal and parietal association areas [34]. It was also shown that brain network patterns associated with motor and cognitive functions are orthogonal of each other [32]. By applying network analysis to the serotonergic system, we can further study the functioning role of serotonergic pathways in PD.

1.3.2 Principal Component Analysis

SSM is based on PCA to decompose sources of variation (deviation) in the data into a set of linearly uncorrelated/orthogonal vectors called Principal Components (PCs). PCs are ranked based on the variance accounted by each PC in the subject by region data matrix, so that the first PC accounts for the largest variance. PCA is done by Singular Value Decomposition (SVD) of the data matrix (detailed mathematical steps are listed in the next subsection) [35].

1.3.3 SSM/PCA Analysis

After minimizing substantive variability in subjects and brain regions, we can identify the significant spatial covariance patterns in the combined control and patient groups. Detailed computational steps of region-based SSM are listed below [30][28]:

- 1. subject PET images are smoothed and normalized onto a common template (e.g. the normalized Talairach-like space (MNI)) using Statistic Parametric Mapping (SPM) software. This step ensures all functional activity measurements at different locations in the brain are mapped onto the same coordinate system in a one-to-one correspondence fashion. (This step can be eliminated when using regional BP_{ND} values).
- regional quantitative measurements (e.g. BP values or SUV) are obtained using pre-defined brain region mask. Value in each region r (1,...,N) of each subject s (1,...,M) are combined together to form the subject by region data matrix P_{sr}.
- 3. (optional) depending on the characteristics of the quantitative measurements, logarithmically transformation of P_{sr} can be applied. Logarithmically transformation makes highly skewed distributions less skewed, and changes multiplicative scaling effect into additive components which can be then removed by double centering step (step 4). For BP data, we did not apply any transformation before applying statistical analysis.

$$P_{sr} \longrightarrow Log P_{sr}$$

4. $LogP_{sr}$ is centered with respect to subject means in each region $LGMR_r$ and region means in each subject GMP_r obtain the Subject Residual Profile (SRP_{sr}) . $LGMR_r$ is the mean across subjects of brain data of region r, and GMP_r is the mean across regions of subject s. Through this double centering process, the resulting matrix SRP_{sr} is the deviation of the mean subject and mean regional values which represents a coordinate system that relates differences from the mean values. Double centering ensures the result was invariant to subject and regional scaling effects.

$$SRP_{sr} = P_{sr} - LGMR_r - GMP_r$$

$$where \ LGMR_r = mean_{region}(LogP_{sr})$$

$$GMP_r = mean_{subject}(LogP_{sr}) - mean_{subject}(LGMR_s)$$
(1.13)

5. Singlular Value Decomposition (SVD) of SRP_{sr} matrix to derive the regional brain patterns and the associated subject scores. We first determine the MxM subject by subject covariance matrix S_{sub} in matrix format as:

$$S_{sub} = SRP * SRP^T \tag{1.14}$$

Eigenvalue decomposition of the matrix S_{sub} results in eigenvalues (λ_k , k=1,...,M) and eigenvectors (e_k , k=1,...,M):

$$S_{sub}e_k = \lambda_k e_k \tag{1.15}$$

After left multiplying both sides by SRP^T :

$$SRP^{T}S_{sub}e_{k} = \lambda_{k}SRP^{T}e_{k}$$

$$(SRP^{T}SRP)SRP^{T}e_{k} = \lambda_{k}SRP^{T}e_{k}$$

$$(1.16)$$

where (SRP^TSRP) is the NxN region by region covariance matrix S_{reg} . We can also get the Group Invariant Subprofile vectors (GIS_k) as eigenvectors of matrix S_{reg} using the same eigenvalues as before λ_k

$$S_{reg}GIS_k = \lambda_k GIS_k$$
where $S_{reg} = SRP^T SRP$ and $GIS_k = SRP^T e_k$

$$(1.17)$$

 e_k vectors weighted by the square root of their corresponding λ_k eigenvalues gives the subject score vectors $(Score_k)$ whose elements represent the pattern vector GIS_k

$$Score_k = \sqrt{\lambda e_k}$$
 (1.18)

So as a result of SUV, SRP_k for each subject is expressed as sum of the GIS_k multiplied by corresponding subject score $(Score_k)$ along each PC:

$$SRP_k = \sum_k Score_k GIS_k \tag{1.19}$$

The eigenvalues represent the Variance Accounted For (VAF_k) for each vector:

$$VAF_k = \lambda_k / (\lambda_1 + \lambda_2 + \dots + \lambda_k) \tag{1.20}$$

Identify Disease-Related pattern

Therefore, for each PC, we have the subject scores for each subject and GIS values for each region. To identify significant topographic covariance profiles which best distinguish between subject groups, PCs related to disease are judged by discriminative accuracy between groups. A single or linearly combination of GIS vectors has to separate the subject scores of two subject groups at a pre-specified statistical threshold. Only the first few PCs accounting for a relatively significant amount of variation in the original data matrix, which correspond to major sources of spatial variance, should be considered. The general steps for identification of significant disease-related spatial covariance pattern are listed below:

- 1. choose PCs accounting for a significant amount of total variation in the data matrix for further analysis. This eliminate PCs accounting for low percentage of total variance due to noise.
- 2. choose PCs satisfying a pre-specified statistical threshold. This is done by applying two-sample T-test on the subject scores along each PC, and select only PCs with p-value lower than a pre-specified threshold (e.g. p < 0.001) to eliminate noise. This threshold is based the characteristics of the data matrix.
- 3. subject scores of the selected PCs are entered into logistic regression models with groups (binary number) as dependent variables and subject scores as



Figure 1.7: Overflow of the Scaled Subprofile Model.

independent variables. The combination of PCs with the lowest Akaike Information Criterion (AIC) [36] is selected as the one which best distinguishes between groups.

- 4. p-value of likelihood-ratio test for this combination of Z-transformed subject scores (based on equation 1.21) is used to examine the level of discrimination between groups.
- 5. PCs are then combined using the coefficients from the regression model to yield a single disease-related covariance pattern

$$Z_{score} = (score - mean(score(1:NC)))/std(score(:))$$
(1.21)

To obtain the most robust results that is most suitable for our dataset, we combined SSM analysis with bootstrap resampling techniques to identify the diseaserelated covariance pattern. Details about the modification can be found in the method section.

Topographic Profile Rating

After obtaining the significant disease-related covariance pattern, we can apply the forward application to calculate the subject scores for a specific pattern on individual basis using equation 1.22. This process is called the topographic profile rating (TPR) [28].

$$SRP_k^T GIS_k = Score_k \tag{1.22}$$

1.4 Parkinson's Disease

1.4.1 Background

Parkinson's disease (PD) is the second most common progressive neurodegenerative disorder of the central nerves system (CNS), and has a prevalence of approximately 0.3% of the entire population. PD affects around 100,000 Canadians with a cost of about \$2.5-5 billion annually. The prevalence of PD increases significantly with age, affecting about 4.4% of people over 50 years of age and 11.9% over 80 years of age [37].

1.4.2 Symptoms

Motor Deficits The most common symptoms of PD are abnormalities in motor system, including resting tremor, rigidity and difficulty initiating and sustaining voluntary movements. These motor abnormalities are known to caused by the presence of Lewy bodies in the brain, which results in the degradation of nigrostriatal dopamine (DA) neurons and therefore alters the activity of the motor corticostriatopallido-thalami cortical (CSPTC) pathways. It is known that the motor symptoms of PD start to occur when about 50% of the nigral dopaminergic neurons have died, resulting in 80% reduction in the striatal DA content. [38]. Pathologic process of PD begins in the dorsal motor nucleus, proceeding to midbrain and forebrain in different stages of the disease as predicted by the Braak hypothesis [39]. This implies the existence of a relatively long preclinical period during which several disease-induced neurochemical changes take place.

Cognitive Deficits Apart from common motor abnormalities, PD patients often experience cognitive deficits which may occur before or along with motor deficit. Some of these deficits, such as depression and dementia, occur in more than one third of PD patients and take years to develop before initial motor symptom onset which make them potential preclinical markers of PD. These motor and cognitive deficits can have a great impact on the quality of life. There is currently no cure for PD, medications and surgeries aim to reduce symptoms and improve quality of life through coping mechanism which help patients adapting to motor and cognitive limitations, mainly by using levodopa or DA agonists. [40] [41] However, treatment-induced cognitive complications are common side effects. [42].

Exact causes of these cognitive symptoms are still unclear. Some studies have suggested that the degradation of nigrostriatal DA content may also contribute to the cognitive deficits in PD due to the direct connections between the ventral tegmental area (VTA) and the prefrontal cortex [43]. However, changes in the dopaminergic pathway alone cannot fully explain the cognitive deficits of the disease. Several studies have suggested that the serotonergic pathway may contribute to several non-motor disturbance.

Treatment There is currently no cure for PD, medications and surgeries aim to reduce symptoms and improve quality of life. The most commonly used medication is the pharmacological replacement of DA, which is mostly accomplished by
levodopa (L-DOPA). L-DOPA is a precursor of DA and is converted to DA in the dopaminergic neurons. Since motor symptoms occur as a result of DA degeneration, administration of L-DOPA temporarily diminishes the motor symptoms. DA agonists can also be used. Agonist binds to DA receptor and activates the receptor to produce a biological response.

Motor function responds well to the DA therapy. The most common complication as a result of DA therapy is dyskinesia, which is involuntary muscle movement and can range from slight tremor of the hands to uncontrollable movement of the body. Treatment of cognitive symptoms in PD mainly aims to reduce symptoms or improve quality of life through coping mechanism which help patients adapting to cognitive limitations [40].

In order to better understand the disease, we will look closer at two neurological pathways inside the brain: dopaminergic pathway which is the most commonly known pathway to be affected by PD and serotonergic pathway which is linked more closely to cognitive abnormalities of the disease.

1.4.3 Dopaminergic System

Motor abnormalities are caused by the presence of Lewy bodies, which results in the degradation of nigrostriatal DA neurons. Pathologic process of PD begins in the dorsal motor nucleus, proceeding to midbrain and forebrain [39].



Figure 1.8: Chemical structure of dopamine molecule

Dopamine (3,4-dihydroxyphenylethylamine) is the neurotransmitter that controls the dopaminergic pathway, which has a characteristic anatomical pattern in the brain. DA is involved in the regulation of locomotor activity, emotion and neuroendocrine secretion. The chemical structure of dopamine is shown in Figure 1.8.



Figure 1.9: Neuronal projections of four DA systems in human brain [9]

There are four central DA pathways which are defined neuroanatomically as shown in Figure 1.9 [10][9].

- 1. The nigrostriatal system projects from the substantia nigra to the caudate nucleus and putamen, where nearly 80% of DA is located here. Destruction of this pathway results in sever motor dysfunction.
- 2. The mesolimbic system originates in the ventral tegmental area of the midbrain and projects to nucleus accumbens, olfactory tubercle, hippocampus, septal nuclei, and amygdala. This system is heavily involved in emotions, memory and the reward system.
- 3. The mesocortical system originates in the ventral tegmental area and projects to the anterior cingulate cortex, septum, neocortex and prefrontal cortex. These cortical regions are important for motivation, cognition and emotional control.
- 4. The tuberoinfundibular system projects from hypothalamus to the pituitary gland, which regulates the neuroendocrine functions.

DTBZ

It is known that the motor symptoms of PD start to occur when about 50% of the nigral dopaminergic neurons have died, resulting in 80% reduction in striatal DA content. We used $[^{11}C]$ -dihydrotetrabenazine (DTBZ) to estimate dopaminergic denervation. DTBZ is a presynaptic vesicular monoamine type 2 (VMAT2) marker, which competes with DA and binds to these VMAT2 DA transporters within DAproducing neurons. VMAT2 binding site is a specific protein located in the membranes of presynaptic vesicles, so DTBZ is used to access membrane DA transporter binding. Even though DTBZ is not specific to DA, it is found that over 95% of VMAT2 binds to DA terminal in the striatum (largely in the storage vesicles in the predominant dopaminergic terminals) and does not undergo any major disease or treatment induced regulatory changes. Studies showed that there is striatal DTBZ uptake reduction is correlated with motor disability and disease progression of PD [44]. The advantage of DTBZ is not up regulated by DA, so there is a consistent rate of decrease in the course of disease progression [45].

1.4.4 DA release

The DA transmission process involves the production, release, reuptake, breakdown and diffusion of DA as shown in Figure 1.10. DA is produced from tyrosine by DOPA decarboxylase (DDC) in the presynaptic neuron. DA is then taken up by storage vesicles which carry and release DA into the synapse. Once in the synapse, DA can react with 5 different post-synaptic DA receptors which trigger various cascade of intracellular signaling in different parts of the brain. For example, D2 receptors are mainly located in the striatum, limbic areas, hypothalamus and pituitary gland [10].

Abnormal DA release in the synapse was shown to relate to treatment-induced motor complications in PD patients [46] due to dramatic changes in receptor occupancy. DA release pattern was analyzed in details using double RAC scans as shown in later sections.

RAC

Radiotracer $[{}^{11}C]$ -raclopride (RAC) is used to study the change in DA release pattern due to various stimuli. RAC is a dopamine D2 receptor antagonist and can be used to evaluate the amount of DA changes and the synaptic DA loss. The antagonist binds to the receptor and competes with DA released due to any stimuli for



Figure 1.10: Systematic diagram of DA synapse showing the production, release, reuptake, break down and diffusion of DA. [10]

binding to these receptors [47]. Therefore, if DA is released because of any stimuli, the ability of RAC to bind to the receptors will be reduced, resulting in a lower RAC BP_{ND} value for scans taken during stimuli. There is evidence that different disease-induced changes in the synaptic DA levels (referred to as DA release pattern) may be related to different manifestation of the disease and responses to therapy [46][47].

Clinically, RAC shows an asymmetric binding in the left and right hemispheres. There is evidence that in advanced PD subjects, improvement in bradykinesia and rigidity scores following DA medication administration significantly correlated with reduction in RAC binding, suggesting an increased DA release into the synapse. As discussed before, L-DOPA treatment, although remains as the most effective treatment of PD symptoms, can lead to motor fluctuations and complications such as L-DOPA-induced dyskinesia (LIDs). Although the origin of such complications is not clear, it is thought that both pre- and post-synaptic DA systems in combination with non-DA systems play an important role in the development of the complications.

1.4.5 Serotonergic System

Serotonin neurons (5-HT) are originated in the dorsal raphe nuclei which are then projected to the basal ganglia (particularly in the striatum) and to the frontal cortex and limbic system as shown in Figure1.12 [48]. Serotonin (5-hydroxytryptamine, 5-HT) is a monoamine brain neurotransmitter. The chemical formula for serotonin is $N_2OC_{10}H_{12}$ and its chemical structure is shown in Figure 1.11. Serotonin is produced from the hydroxylation and decarboxylation of the amino acid tryptophan. In the central nervous system, serotonin is only synthesized by the neurons of Raphe nuclei which are distributed along the length of the brainstem in nine pairs. Serotonin is widely distributed in the brain and serves an important role in the regulation of mood, sleep, appetite, memory, learning, etc. Low level of serotonin is associated with depression, bipolar disorders, fear and anxiety, since serotonin is required for the metabolism of stress hormones [11].

The serotonergic system is involved in different types of psychopathlogical conditions associated with PD, especially depression, weight and appetite problems [49][50]. The degeneration of serotonergic terminals occur earlier in the disease compared to dopaminergic system. PD patients exhibit progressive, nonlinear loss of serotonergic function, which starts in the caudate, thalamus, hypothalamus and anterior cingulate cortex and expands to the basal ganglia and limbic system as disease progresses [51].

It was shown that 5-HT neurons share the same monoamine biosynthetic components with DA neurons, which contributes to DA processing in denervated striatum in PD subjects [52] and play a role in levodopa-induced dyskinesia (LID) by releasing DA as a false neurotransmitter. An autoradiographic study has shown a higher SERT level in the putamen of dyskinetic than non-dyskinetic levodopa-treated subjects [42].



Figure 1.11: Chemical structure of serotonin

DASB

To study the brain serotonergic system, we used second generation $[^{11}C]$ -DASB PET imaging tracer to measure the level of serotonin transporter (SERT) binding and to estimate serotonin neuronal integrity. DASB has high specificity and sensitivity for SERT, and low affinity for DA transporter (DAT). Studies have suggested a decrease in striatal 5-HT [48] and some observed a striatal hyperinnervation in PD postmortem study and animal models.

1.5 LRRK2 Mutation

The human leucine-rich repeat kinase 2 (LRRK2) gene was discovered in 2004 and is the greatest known genetic contributor to PD. Majority of PD is sporadic PD, meaning the cause of the disease is unknown. About 10 % of the disease is related to genetic mutation in the LRRK2 gene. In the US, LRRK2 mutation accounts for approximately 0.5% of simplex PD (e.g. single occurrences in a family) and 2%-6% of familial PD. [53]

Clinical characteristics of sporadic PD and LRRK2 mutation associated PD patients are quite similar. The motor symptoms are comparable between the two PD groups. Cognitive impairment does not appear to be more common in LRRK2 associated PD than in typical sporadic disease [54].

Studies have shown that there is an increased DA turnover and increased SERT binding in asymptomatic LRRK2 mutation carriers compared to healthy controls



Figure 1.12: Serotonin projections in the brain. Serotonin is produced in nucleus raphe and projects onto other brain regions [11]

[55][56]. Studying LRRK2 associated PD in comparison with healthy and sporadic PD populations can help us to further understand the contribution of genetic mutations to the disease, and understand the presymptomatic stages of the disease.

1.6 Research Objectives

PET imaging has been an effective tool to study altered neurological pathways in patients with Parkinson's disease. Even though motor symptoms of this disease are mainly due to the loss of neurons in the dopaminergic pathway, interactions between the dopaminergic and serotonergic pathways may contribute to treatment-induced complications (motor and cognitive) in later stages of the disease.

The overall objective of this thesis work is to investigate the interactions between the dopaminergic and serotonergic pathways in presymptomatic and early stages of the disease, and their contributions to treatment-induced complications and nonmotor symptoms in PD subjects.

In the first part of the project (Chapter 4), we applied a regional covariance

pattern analysis to [¹¹C]-DASB data in PD subjects (in early and moderate stages). Regions with relatively preserved binding in the disease-specific networks may act as a compensatory mechanism for the dopaminergic system. We also applied the same analysis to asymptomatic LRRK2 mutation carriers to investigate if there is a distinct mutation-specific network, which may explain the increased risk of PD in this group of subjects and maybe used as an earlier marker before motor symptom onset.

In the second part of the project (Chapter 5), we analyzed the medicationinduced DA release pattern on early PD subjects (less than 5 years of disease duration) using double [¹¹C]-RAC scans. We want to examine if altered DA release pattern in response to treatment would contribute to treatment-induced motor complications.

The combination of abnormal medication-induced DA release pattern and upregulation of the serotonergic system may be able to explain the occurrence of treatment-induced complications and non-motor symptoms in PD patients, and act as a potential early marker for the disease.

Chapter 2

Methods

2.1 Subjects and Clinical Information

Subjects We studied 18 non-demented patients with PD, 9 asymptomatic LRRK2 mutation carriers (LRRK2-AMC), and 9 healthy control volunteers (HC). The 18 PD subjects were further divided into 12 sporadic PD (sPD) and 6 LRRK2 mutation-associated PD (LRRK2-PD) subjects. All subjects in this study had no clinical history of depression or had received anti-depressant therapy, and they had no other medication with known action on the serotonergic system. All healthy controls had no history of neurological or psychiatric disorders and were not on any medication. All groups were matched for age and sex (Table2.1).

	Control	sPD	gPD	Unaffected
No of Subjects	9	13	8	9
Age (years)	56 ± 14	59 ± 8	66 ± 15	48 ± 10
Disease Duration (years)		3±3	8±7	
UPDRS total off		16±7	22 ± 10	
UPDRS total on		10 ± 6		
Gender		8M/5F	3M/5F	6M/6F
Tracers	DASB	DASB/DTBZ/MP/RAC	DASB/DTBZ/PBR/PMP	DASB/DTBZ/PBR/PMP

Table 2.1: Participants clinical information: values reported as mean±standard deviation; Disease duration has been accounted from the time of PD motor symptoms initiation (not from time of clinical diagnosis); M=Males; F=Females; UP-DRS=Unified Parkinson's Disease Rating Scale; UPDRS off=UPDRS without drug (LDOPA) intervention; UPDRS on=UPDRS when on drug medication.

All subjects were used in the DASB SSM pattern analysis study. Only sPD subjects were involved in DA release study because only they had double RAC scans to evaluate DA release patterns.

Clinical Evaluation PD subjects were clinically evaluated with the Unified Parkinson's Disease Rating Scale (UPDRS). UPDRS was used to measure severity of motor symptoms and therefore monitor disease progression. UPDRS off was measured when subjects were taken off LDOPA medication; UPDRS on was measured when subjects were still on medication. By calculating the change in UPDRS with and without medication, we can relate the amount of DA released with the effectiveness of the medication.

Age of disease onset for PD patients was defined as reported by the patients, not at diagnosis. Montreal Cognitive Assessment (MoCA) scores were also recorded to access mild cognitive dysfunction. The assessment tests on attention, executive functions, memory, language, visuoconstructional skills, conceptual thinking, calculations, and orientation. Hoehn and Yahr scale was used to access the disease symptoms progression. Beck depression inventory was used to access the severity of depression.

2.2 Scanning Protocol and Image Processing

2.2.1 Scanning Protocol

To perform [¹¹C]-DASB PET scans, a mean dose of 555Mbq of DASB radiotracer was administered by intravenous injection over 60 seconds, and acquisition time of 80 minutes was used. For DTBZ and RAC scans, 60mins scan time was used. PET images were obtained on a high resolution research tomography with an inplane resolution of 2.3mm. Patients stopped medication for at least 18 hours before scanning. MRI scans were performed as resting-state MRI at UBC 3 Tesla MRI center.

2.2.2 Image Processing

Reconstructed images were summed to create the entire dynamic set using Matlab based Statistical Parametric Mapping (SPM12) software. Pre-defined high-contrast region-of-interest (ROI) templates were developed in Montreal Neurological Institute (MNI) space using MRI and PET data from healthy controls.

Subject PET images were coregistered to the corresponding MRI images using SPM12 software, and then warped onto the MNI space to obtain the corresponding transformation matrix. Inverse transformation was applied to the MNI space ROIs to place ROIs onto the original PET images.

ROI Selection

For DASB, ROIs were manually defined on both hemispheres for 21 non-overlapping ROIs which are known to be related to the serotonergic system: anterior and posterior cingulate (ACC and PCC), amygdala, caudate, cerebellum, dorsolateral prefrontal cortex (DLPFC), hypothalamus, insula, medulla, midbrain, orbital frontal cortex (OFC), pons, pedunculopontine nucleus (PPN), putamen, substantia nigra (SN), thalamus, ventral striatum (VS), hippocampus, ventral tegmental area (VTA), dentate nucleus (DN), and globus pallidus (GP).

ROIs for DTBZ and RAC scans were placed on the ventral and dorsal striatum regions (1 on caudate and 3 on putamen) for both hemispheres. Four consecutive saggital slices (17mm) were selected for data extraction for the dorsal striatum regions, 3 consecutive saggital slices (7.5mm) were used for the ventral striatum data extraction. Occipital Cortex was used as the reference region which was defined by 3 ROIs placed on the same slices as the striatum regions for DTBZ. The Cerebellum was used as the reference region which was defined by a single ROI placed on 3 consecutive saggital slices (12.75mm)

Quantitative Measurements

For DTBZ and RAC, Logan plot method was used to obtain BP_{ND} values in each of the 5 ROIs.

For DASB, we obtained 3 sets of quantitative data from these PET/MRI images: regional non-displaceable binding potential (BP_{ND}) values, parametric BP_{ND} values, and standard uptake values (SUV) for all subjects:

- regional BP_{ND} values in each ROI were obtained using Simplified Reference Tissue Model (SRTM) with cerebellum as reference region
- parametric BP_{ND} values were obtained using two-step Reference Tissue Model (SRTM2), which used k2' values generated from RTM (k2'=0.05 min^{-1} for DASB) in the first step and fixed k2' value in the second step
- regional SUV obtained over the last 30mins (50-80mins) time frame

To reduce noise in the network analysis, BP_{ND} values instead of SUVs were chosen as the quantitative measurement of choice. In the next chapter, we compared these quantitative measurements for DASB tracer to 1) validate DASB parametric BP_{ND} algorithm and 2) to choose the best quantitative measurement for network analysis.

Chapter 3

DASB Parametric Validation

In this chapter, we validated the SRTM2 method for DASB tracer by comparing global k2' values in the first step of SRTM2 with reported k2' values in literature and comparing parametric BP_{ND} values with regional BP_{ND} values obtained using SRTM.

3.1 Methods

To calculate parametric BP_{ND} values for DASB data using SRTM2, we need to fix a global k2' value for the second step of the kinetic model as discussed previously. There are two ways to find the global k2' value:

- k2' value for each pixel of the brain image was estimated using SRTM first and then averaged outside the reference region to obtain the global k2' value for each subject [25]
- set k2' value based on literature, which was reported to be $0.056min^{-1}$ for DASB tracer from one-tissue-compartment kinetic modeling [57] [26] [58]

To validate the parametric BP_{ND} values, we first compared the k2' values obtained from the first step of SRTM for 7 healthy control subjects to check the variation and agreement of the calculated k2' values with reported values. In the second step of SRTM2, after fixing k2', BP_{ND} values were estimated using twoparameter fit. We then compare the regional BP_{ND} values obtained from SRTM with the averaged parametric BP_{ND} values in each ROI.

3.2 Results

3.2.1 k2' Values

The calculated k2' values for 7 control subjects had an average of $0.052 \pm 0.017 min^{-1}$ as shown in Figure 3.1. The calculated k2' values agree with the reported true

k2' value $0.056min^{-1}$ from one-tissue-compartment kinetic parameter values using cerebellum as the reference region [57].



Figure 3.1: estimated k2' values for each subject compared to reported k2' value as shown with red line

3.2.2 BP_{ND} Values

To validate SRTM2 parametric BP_{ND} values, we compared the regional values obtained from SRTM and averaged values from SRTM2 in each ROI as shown in Figure 3.2, and averaged regional and parametric values for each subject as shown in Figure 3.3.

When correlating regional BP_{ND} from SRTM and SRTM2, we found a significant correlation between average SRTM BP_{ND} values and averaged parametric BP_{ND} values in each brain region (R2=0.967) as shown in Figure 3.4.

3.3 Discussion

k2' values from SRTM2 showed excellence agreement with reported k2' value and averaged parametric BP_{ND} values also showed excellent agreement with regional BP_{ND} values from SRTM. Parametric BP_{ND} values can be used for network analysis



Figure 3.2: Averaged BP_{ND} values from SRTM and SRTM2 in each ROI

at voxel level and to explore tracer gradient inside a ROI. However, to reduce noise, network analysis in this project focused on 20 pre-defined ROIs instead of individual voxel. The analysis can be extended to voxel level in the future.



Figure 3.3: Averaged BP_{ND} values from SRTM and SRTM2 in each subject



Figure 3.4: Correlation between SRTM and SRTM2 BP_{ND} values in each ROI. Equation of the linear regression model is shown in the Figure

Chapter 4

SSM Pattern Analysis in Serotonergic System

4.1 Methods

To identify network abnormalities, we used a region-based network modeling approach, the Scaled Subprofile Model (SSM), defined previously [30]. This approach is based on multivariate principal component analysis (PCA) and models the sources of subject and region variation as spatially distributed networks in functional images [28]. SSM is able to identify a group-dependent, region-specific, disease specific spatial covariance patterns in the brain that can be used study the heterogeneous regional interactions in different patient groups and to discriminate patients from healthy controls.[28] [29]

In this study, we applied SSM on BP_{ND} values in 20 brain regions to identify significant regional covariance networks which best distinguish between subject groups based on individual expression of the principal components (PC).

4.1.1 Pattern Identification

To limit the analysis to a subset of PCs related to the disease, individual PCs must account for at least 5% of the total subject by region variability in the data. To improve the stability of the selected PCs and the estimated weight for each PC in the logistic regression model, bootstrap resampling was performed 1000 times to obtain the frequency histogram of PCs entering the regression model.

In the case of identifying disease-related pattern using HC and PD subjects, only 7 out of 28 PCs made the 5% cutoff and were used to generate the PC histogram (Fig4.1). The scree test was then used to determine the frequency cutoff to choose the optimal number of PCs to include in the model.

After selecting the subset of PCs, frequency histograms of the estimated model



Figure 4.1: Frequency Histogram of the included PCs. Only 7 PCs account for greater than 5% of total variance in the data. The first 3 PCs with the highest frequency were entered into the logistic regression model to obtain the combined disease-related pattern.

parameters of the selected PCs were used to determine the weights of each PC. Subject scores for each selected PC were then entered singly or in linear combination into a forward stepwise logistic regression models (Matlab scripts, Mathworks). The combination of PCs with the lowest Akaike Information Criterion (AIC) score [36] was selected to yield a single disease/mutation-related covariance pattern. This model was used to estimate the corresponding weights (coefficients) on each pattern, that in linear combination, best discriminated between subject groups. In this case, histograms of model coefficients of the selected PCs gave the weights of 0.174, 0.267 and 0.232 for PC2, PC4 and PC5 respectively.

The final disease/mutation-related spatial covariance pattern was a linear combination of the regional weights of the selected PCs. The same coefficients were then applied to subject scores for the three PCs to compute the combined SPDRP subject score for each individual subject. Regional weights for this specific combination of PCs were Z-thresholded at 1 to select significant regions contributing to the corresponding covariance pattern. Network expression for new subjects or test set in validation was computed using the projection of the subject data onto the corresponding spatial maps. This process, the topographic profile rating (TPR), was defined previously.



Figure 4.2: Frequency Histogram of the model parameters of the included PCs in the logistic regression model. The mean of each frequency histogram was used as the coefficient for each corresponding PC in the logistic regression model.

4.1.2 Validation

To validate the disease/mutation-related spatial covariance pattern, we preformed 5-fold cross-validation with 1000 iterations. All subjects in the analysis were divided into 5 groups; 4 groups were used as training set to obtain the pattern; 1 group was used as test set to examine the robustness of the pattern by calculating individual subject expression of this specific pattern. The sensitivity and specificity of each discrimination were determined using Receiver Operating Curve algorithm (ROC).

4.2 Results

4.2.1 Absolute BP_{ND} Values

Before applying PCA to the data, we performed group analysis on the mean BP_{ND} values in all 20 regions in all 4 subject groups to validate the choice of the ROIs and get a sense of possible regions might appearing in the covariance brain pattern.

Looking at BP_{ND} values, there was a significant decrease in BP_{ND} values in caudate, amygdala and putamen in PD compared to HC subjects. PD subjects showed a lower BP_{ND} in all 20 regions compared to HC subjects, but there is a relatively smaller decrease in hypothalamus compared to other regions. There was a significant higher BP_{ND} values in ACC, amygdala, hypothalamus and medulla in the asymptomatic LRRK2 mutation carrier (LRRK2-AMC) subjects compared to HC subjects. There was no significant difference between LRRK2-associated PD (LRRK2-PD) and sporadic PD (sPD) in any brain region.

There was a significant age correlation in left hypothalamus, left amygdala and right PPN regions in HC BP_{ND} values. We did not observe any significant cor-





Figure 4.3: Serotonergic Parkinson's disease-related pattern (SPDRP) identified by spatial covariance analysis of DASB PET scans from 17 PD patients. This pattern was characterized by a relative decreased SERT binding in caudate and putamen, and a covarying increased SERT binding in hypothalamus, hippocampus, anterior cingulate (ACC), amygdala, and medulla. Only regions that significantly contributed to the network at Z>1. Regions with positive weights (increased binding) are colour-coded red; those with negative weights (decreased binding) are colour-coded blue.

relation between BP_{ND} values and age or age of onset in any brain region in PD subjects. With age as a covariate, there was a significant group differences in amygdala (p=0.008), caudate (p=0.011), putamen (p=0.043), hypothalamus (pi0.001) and medulla (p=0.005) between LRRK2-AMC and HC subjects.

4.2.2 PD vs HC

Disease-specific spatial covariance pattern was derived using BP_{ND} values in 20 brain regions from 9 HC and 18 PD subjects. Subject scores significantly separated HCs from PD patients (p<0.0001). The pattern was obtained from PC2, PC4 and PC5, which accounted for 27% of the total variance in the subject by region BP_{ND} data set. The serotonergic Parkinson's disease-related pattern (SPDRP) was characterized by a relative decreased SERT binding in caudate and putamen, and a covarying increased SERT binding in hypothalamus, hippocampus, anterior cingu-



Figure 4.4: SPDRP expression in HC, PD and LRRK2-AMC subjects. There was a significant separation between HC (blue) and PD (red) groups (pj0.0001). LRRK2-AMC subjects did not show an elevation of this disease-specific pattern.

late (ACC), amygdala, and medulla. Only regions that significantly contributed to the network at Z>1.

Correlation with Clinical Measurements

There was an almost significant negative correlation between SPDRP expression (subject scores) and age of onset (p=0.0579) as shown in Fig.4.5. There was also an almost significant positive correlation between SPDRP expression and UPDRS motor scores (p=0.0558) as shown in Fig.4.6. No correlation was observed for disease duration or age.

SPDRP Expression in Asymptomatic LRRK2 Mutation Carriers

LRRK2-AMC subjects did not show an elevated expression of SPDRP compared to HC subjects (p=0.14).

4.2.3 Asymptomatic LRRK2 Mutation Carrier vs HC

Asymptomatic LRRK2 mutation-related spatial covariance pattern (LRRK2-AMRP) was derived using BP_{ND} values in the same 20 brain regions from 9 HC and 9 asymp-



Figure 4.5: SPDRP expression vs age of disease onset in PD subjects. SPDRP expression in PD subjects showed an almost significant correlation with age of onset (p=0.0579).



Figure 4.6: SPDRP expression vs UPDRS motor score in PD subjects. SPDRP expression in PD subjects showed an almost significant correlation with UPDRS (p=0.0558).

tomatic LRRK2 mutation carriers (LRRK2-AMC). Subject scores were significantly higher in LRRK2-AMC subjects compared to HC (p<0.0001). The pattern was obtained from PC3, PC1, PC2 and PC5. The resulting LRRK2-AMRP was comprised of a relatively decreased binding in putamen, and relatively preserved binding in hypothalamus, amygdala, PPN, midbrain, substantia nigra (SN) and medulla.





Figure 4.7: Serotonergic asymptomatic LRRK2 mutation-related spatial covariance pattern (LRRK2-AMRP) identified by spatial covariance analysis of DASB PET scans from 9 LRRK2-AMC subjects. This pattern was characterized by a relative decreased SERT binding in putamen, and a covarying increased SERT binding in hypothalamus, amygdala, midbrain, PPN, SN and medulla. Only regions that significantly contributed to the network at Z>1. Regions with positive weights (increased binding) are colour-coded red; those with negative weights (decreased binding) are colour-coded blue.



Figure 4.8: LRRK2-AMRP expression in HC, PD and LRRK2-AMC subjects. There was a significant separation between HC (blue) and PD (red) groups (pj0.0001). LRRK2-AMC subjects did not show an elevation of this disease-specific pattern.

LRRK2-AMRP Expression in PD subjects

PD subjects did not show an elevated expression of LRRK2-AMRP compared to HC subjects (p=0.65).

Validation

We preformed 5-fold cross validation with 1000 iterations to confirm the obtained pattern. In each iteration, subjects were divided randomly into five subsets: four subsets were used to train the model to obtain the covariance pattern, and the remaining one group with each subset containing approximately equal number of members from different groups was used to test the accuracy of the classification.

Receiver Operator Curve (ROC) was used to examine the accuracy and specificity of the pattern. Area under curve (AUC) for training set is 0.98 and 0.62 for testing set for the PD vs HC pattern (SPDRP).



Figure 4.9: ROC for training set for 1000 iterations.



Figure 4.10: ROC for testing set for 1000 iterations

4.3 Discussion

In this study, we applied network analysis to DASB PET data from sporadic PD (sPD), LRRK2-associated PD (LRRK2-PD) and asymptomatic LRRK2 mutation carriers (LRRK2-AMC) to identify a novel disease or mutation-related spatial co-variance pattern in the serotonergic pathways.

4.3.1 Use of PCA to Identify Disease/Mutation-Related Topographies

Functional imaging techniques allow us to quantify brain activity to study pathophysiology of neurodegenerative disorders, but absolute tracer binding or metabolic activity may not provide the complete picture. In addition, variability in subjects and brain regions increases the difficulty in quantitative analysis. Comparing to traditional group analysis using BP_{ND} amplitudes, pattern analysis is proven to be more robust and sensitive to small change in brain physiology.

Studies have suggested that cognitive processes depend on interactions among distributed brain regions, which are characterized by brain connectivity [59]. The scaled subprofile model (SSM) employed in this analysis was able to examine the subject by region interactions in SERT binding, while eliminating global and regionspecific effects in the data. Before applying PCA, BP_{ND} data was double-centered to obtain the residual regional BP_{ND} values which contain relevant biological information independent of the global mean.

A more detailed review of the mathematical principles and basic assumptions underlying this method was discussed previously [60].

4.3.2 ROI-based Network Analysis

All the 20 ROIs in this analysis were chosen based on prior knowledge about the serotonergic system. Choice of ROIs were also confirmed by analyzing the absolute BP_{ND} values between groups. In this project, network analysis was done on ROI-level only to 1) reduce noise compared to BP_{ND} values and 2) compare to absolute regional BP_{ND} values. Future analysis can be extended to voxel level when no prior knowledge or hypothesis is present.

4.3.3 PC1

For FDG PDRP, the PC1 was found to best separate between subject groups and no other PCs were entered into the logistic regression model. In our case, PC1 was mainly contributed by the noise in the ROIs. Regions contributed the most to PC1 included midbrain, PPN, SN and pons, which were also shown to have higher noise in raw data compared to other regions. TACs in these regions have the largest variation, which results in large variance in BP_{ND} in these regions.

4.3.4 Pattern Identification and Validation

ROIs contributing to SPDRP or LRRK2-AMRP were selected based on a pre-defined threshold (Z-transformed regional weights are greater than 1). We note that contributions from two hemispheres were asymmetrical in some regions, with relatively greater involvement of one hemisphere or the other. This is likely attributed to the noise in the BP_{ND} values before the PCA analysis.

Validation

In 5-fold cross validation, accuracy for test set is not optimal (AUC=0.62). This relatively low AUC was mainly due to high false positive rate in the classification of HC subjects. However there is good accuracy in the classification of PD subjects. Due to the imbalance in the number of HC and PD subjects and the low number of subjects in the analysis, the logistic model can suffer the overfitting problem. Ways to improve AUC for the test set include 1) reducing the number of PCs included in the regression model, 2) balancing the number of subjects in two groups, and 3) including more subjects into the analysis.

4.3.5 Comparison with Other Network Analysis

For PD subjects, they showed a relatively decreased SERT binding in caudate (most significant) and putamen, and relatively preserved SERT binding in hypothalamus (most significant), hippocampus, medulla, dentate nucleus, anterior cingulate and amygdala compared to HC. Here, we compared SPDRP obtained from PD vs HC subjects to common network analysis to examine if there is any similarities between these networks.

FDG/PET PDRP and PDCP

PCA-based covariance analysis has been previously applied to FDG/PET data as discussed before in the Introduction Chapter. The Parkinson's Disease Related Pattern (PDRP) and Parkinson's Disease Cognitive Pattern (PDCP) were validated across different patient populations.

PDRP as previously defined using FDG PET was characterized by hypermetabolism in the thalamus, globus pallidus (GP), pons, and primary motor cortex, with associated relative metabolic reduction in the lateral premotor and posterior parietal areas. PDCP defined also with FDG PET was characterized by relatively increased activity in the cerebellar vermis and dentate nuclei, with associated decreased activity in frontal and parietal association areas [30].

No direct correlation was found between SPDRP and FDG PDRP or FDG PDCP. We do not expect to see a close connection between these distinct patterns, because DASB specifically targets the serotonergic pathway while FDG targets brain metabolic activities.

Resting-State fMRI Connectivity

SPDRP also did not resemble any of the known resting-state fMRI connectivity networks. This is also expected since resting-state fMRI looks at brain activation instead of any specific neurological pathways in the brain.

However, by applying network analysis (such as Independent Component Analysis (ICA))) to resting-state fMRI images for the same subjects, we can incorporate the serotonergic network with functional activation network. Advance statistical analysis (such as joint ICA) can be applied to both DASB and resting-state fMRI data together to examine the intrinsic network underlying both modalities.

4.3.6 Possible Functional Basis for SPDRP and LRRK2-AMRP Topography

In this section, we look into the regions involved in SPDRP and LRRK2-AMRP and try to link them with their functional roles in the brain related to the disease.

Regions with Decreased Binding

Compared to the localized SERT binding reduction (as measured by absolute BP_{ND} values in each ROI), PD subjects showed a significantly lower SERT binding compared to HC subjects in caudate and putamen, which were also shown to have a relative decreased binding in SPDRP.

Previous studies showed that PD subjects had a significant decreased absolute SERT binding in caudate (30%) and putamen (26%) compared to HC subjects [61] [62]). Unlike in the dopaminergic system where posterior putamen is more affected by the disease, there is a preferential loss of SERT in caudate [62] which is consistent with the greater relative decreased binding in caudate compared to putamen in SPDRP.

Regions with Increased Binding

Regions with significantly higher absolute BP_{ND} values (ACC, amygdala, hypothalamus and medulla) in LRRK2-AMC compared to HC subjects all showed a relative preserved binding in SPDRP. This may indicate that upregulated regions in SP-DRP are affected before motor symptoms onset and may act as a compensatory mechanism in the serotonergic system.

According to Braak hypothesis of PD brain pathological staging, Lewy body and neurite deposition occur in raphe nuclei (in brainstem) at stage 2; substantia nigra (SN) and midbrain are affected at stage 3 where clinical motor symptoms start to occur [39].

Both SN and midbrain showed upregulated binding in LRRK2-AMRP, but not in SPDRP. One study suggested SERT loss in striatum regions of PD precedes the loss in midbrain region [63], which may indicate the upregulation in midbrain and SN tries to compensate the loss of SERT in the striatum regions in the presymptomatic stage of the disease.

Regions with relative preserved binding were also shown to be involved in cognitive impairments of PD. For PD patients with depression, hypothalamus and amygdala showed a higher SERT binding than PD patients without depression [?][49]. These two regions both showed upregulated binding in SPDRP and LRRK2-AMRP. PD patients with abnormal BMI changes showed significantly higher SERT binding in hypothalamus compared to cases with no significant BMI changes [50]. These findings may suggest upregulation of SERT function starting from presymptomatic stages of the disease may be related to depression and BMI changes in PD patients.

Comparing SPDRP with LRRK2-AMRP, putamen was the only region with relative decreased binding in LRRK2-AMRP. This may indicate that even though caudate is more affected after motor symptom onset, putamen is still affected earlier by the disease in the presymptomatic stage.

In the presymptomatic LRRK2-AMRP, there are more regions with relatively increased binding than in SPDRP. This may indicate a stronger upregulation in the presymptomatic stage compared to symptomatic stage of the disease. This stronger upregulation may act as a compensatory mechanism in the serotonergic system, but this over activation of serotonergic system may lead to various non-motor symptoms after disease onset.

4.3.7 Correlation with Clinical Measurements

We observed an almost significant correlation between SPDRP expression and UP-DRS motor scores, which indicate more sever patients (in terms of motor disability) may have a higher expression of SPDRP.

We also observed an almost significant negative correlation between SPDRP expression and age of disease onset, meaning that younger onset patients have a higher expression of SPDRP. This finding can be combined with the DA release findings discussed in the next chapter to examine the possible relationship between serotonergic network.

Chapter 5

Levodopa-Induced Dopamine Release in PD

In the second part of the project, we analyzed the drug-induced DA release pattern on early PD subjects (less than 5 years of disease duration) using double RAC data. The combination of abnormal drug-induced DA release pattern and upregulation of the serotonergic system may be able to explain the occurrence of treatment-induced complications in PD patients.

5.1 Objectives

In this study, we investigated the levodopa (LDOPA)-induced DA release pattern for 10 early (less than 5 years disease duration) sporadic PD subjects using double $[^{11}C]$ -RAC scans to examine the relationships between DA release pattern, DA denervation and SERT binding.

Since we already observed some regions with upregulated SERT binding in PD subjects, we want to investigate if the combination of abnormal drug-induced DA release pattern and upregulation of the serotonergic system may be able to explain the occurrence of treatment-induced complications in PD patients.

5.2 Methods

5.2.1 Subjects and Quantitative Measurements

We examined the correlations between LDOPA-induced DA release (as measured by double RAC scans), DTBZ BP_{ND} values and DASB BP_{ND} values for 10 early sporadic PD subjects.

DA release was calculated as RAC BP_{ND} values without drug minus RAC BP_{ND} values with drug. Unified Parkinson's Disease Rating Scale (UPDRS) was measured

No.Subjects	Age	Disease Duration	UPDRS on drug	UPDRS off drug
10	57.8 ± 8.7 years	2.1 ± 1.4 years	10.8 ± 5.4	13.7 ± 4.7

Table 5.1: Clinical information for 10 early sporadic PD subjects

with and without drug to evaluate the severity motor symptoms in each subject. The change in UPDRS was defined as the difference between UPDRS off and on drug, which should correspond to the effectiveness of the drug on individual subject.

 BP_{ND} values were extracted in 3 striatum regions (caudate, ventral striatum and putamen) using either Logon or RTM method with cerebellum used as the reference region. The 3 striatum regions were further divided into the most and least affected sides for each subject, which was defined using the averaged DTBZ BP_{ND} in the putamen regions (i.e. the side with lower DTBZ putamen BP_{ND} had severer degradation of dopamine-producing neurons, so was defined as the most affected side).

5.2.2 Regression Models

We used linear and multiple regression analysis to examine the relationship between DA release, DTBZ BP_{ND} values, DASB BP_{ND} values and clinical measurements in each striatum region. Detailed regression method is as follows:

- 1. DA release values were regressed on each of the possible explanatory variables (predictors) separately (linear regression)
 - DTBZ BP_{ND}
 - DASB BP_{ND}
 - RAC baseline BP_{ND}
 - Age of onset
 - Change in UPDRS (UPDRS off drug UPDRS on drug)
 - disease duration
 - gender
- 2. Multiple regression was applied on all predictors which survived a cut-off criterion from the linear regression
 - Cut-off criterion: correlation p-value < 0.3

Correlations between DA release and all the above variables were examined in each of the striatum regions for all subjects.

5.3 Results

5.3.1 Correlations in Striatum Regions

Most Affected Putamen

In the most affected putamen region, DA release showed a significant correlation with age of onset and change in UPDRS (p=0.013 and p=0.049) in linear regression as shown in Figure 5.1 and Figure 5.2. No variable was significant in multiple regression after correcting for other variables.



Figure 5.1: Correlation between DA release and age of onset for sPD subjects in most affected putamen region.

Least Affected Putamen

In the least affected putamen region, DA release correlated significantly with age of onset and RAC baseline BP_{ND} values (p=0.030 and p=0.012). Age of onset and RAC baseline BP_{ND} values increased significance level when entered in multiple regression (p=0.00060 and p=0.00029) after correcting for each other as shown in Figure 5.3 and Figure 5.4.



Figure 5.2: Correlation between DA release and change in UPDRS for sPD subjects in most affected putamen region.



Figure 5.3: Correlation between DA release and RAC baseline BP_{ND} values after correcting for age of onset for sPD subjects in least affected putamen region.

Caudate

No correlation was found between DA release and any possible explanatory variables. However, there was a significant correlation between RAC baseline BP_{ND} values and age of onset for both the most and the least affected Caudate (p=0.0077 and p=0.015) as shown in Figure 5.5.



Figure 5.4: Correlation between DA release and age of onset after correcting for RAC baseline BP_{ND} values for sPD subjects in least affected putamen region.



Figure 5.5: Correlation between RAC baseline BP_{ND} values and age of onset for sPD subjects in caudate region.

5.3.2 Disease Severity

Disease severity can be defined by either UPDRS off medication or DTBZ BP_{ND} values. DA release did not correlate with either disease severity measures.

In the 10 early PD patients, UPDRS off drug did not depend on age (p=0.067) or age of onset (p=0.12). DA depletion (defined by DTBZ BP_{ND} values) did not depend on age (p=0.66), age of onset (p=0.8), UPDRS off (p=0.52), change in UPDRS or disease duration.

5.3.3 Correlation with Other Tracers

No significant correlation was found between DA release and DTBZ or DASB BP_{ND} values in any of the striatum regions.

5.4 Discussion

5.4.1 Correlations with Disease Severity

Studies have shown that age of onset did not influence the absolute severity of nigrostriatal damage as measured by DTBZ BP_{ND} values [46][65], which agrees with the fact that we did not see any significant correlation between DTBZ BP_{ND} values and age or age of onset in early PD subjects.

5.4.2 DA Release Correlation with Age of Onset and Change in UPDRS

Our results suggest that, for early PD patients, younger onset patients have an increased DA release in response to LDOPA stimuli and a trend towards better motor response to the medication. This was indicated by a strong negative correlation between DA release and age of onset in the putamen region after adjusting for RAC BP_{ND} at baseline, and a trend of positive correlation between DA release and change in UPDRS motor scores.

Younger onset patients have a higher DA release while motor symptoms severity (as measured by UPDRS motor scores) and DA depletion (as measured by DTBZ BP_{ND} values) remain relatively the same compared to older onset patients. This finding implies that DA release in younger onset patients undergoes larger alteration, which results in larger swing in synaptic DA levels. This large swing or imbalance may contribute to greater risk of motor fluctuations, which may explain age-dependent occurrence of complications. We are following these PD subjects clinically (follow-up 3 years after first scan) to see if they would develop treatmentinduced motor complications.

These results will be validated by including more subjects into the study.

5.4.3 Relationship with Serotonergic System

As shown before in Chapter 4, there was an almost significant negative correlation between SPDRP expression and age of onset for PD subjects.

5-HT terminals also participate in DA re-uptake (Berger 1978) and can metabolize L-DOPA into DA. DA released in the striatum by 5-HT terminals acts as false neurotransmitter which contributes to L-DOPA-induced dyskinesia (LID). The decline in SERT levels i the striatum of PD precedes the decline in midbrain and other regions, so we see a relative preserved binding in some brain regions. The upregulation in these serotonergic projection regions may act as a compensatory mechanism in the serotonergic pathway for the loss of dopamine-producing neurons in early stages of the disease.

Chapter 6

Conclusions and Future Work

In this project, we used a PCA-based network analysis to investigate whether a disease or a LRRK2 mutation specific spatial covariance pattern exists in the serotonergic system. A disease-related SPDRP and a mutation-related LRRK2-AMRP were found. Brain regions with a relatively preserved binding may act as presymptomatic marker and/or compensatory mechanism for the disease, which also may link to nonmotor symptoms of the disease. We also investigated the altered medication-induced DA release pattern in early PD subjects. The combination of abnormal medicationinduced DA release pattern and upregulation of the serotonergic system may be able to explain the occurrence of treatment-induced complications and non-motor symptoms in PD patients, and act as a potential early marker for the disease.

As discussed before, more subjects will be included in the analysis to confirm the results and improve statistical power. Patients involved in DA release study will have follow-ups to check if medication-induced motor complications occur. Network analysis can also be extended to voxel level after reducing noise in the parametric BP_{ND} images. Other network analysis can also be applied to combine different imaging modalities (PET and fMRI) or different PET tracers together to gain better understanding of the disease.
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