ROI-based Brain Functional Connectivity Using fMRI: Regional Signal Representation, Modelling and Analysis

by

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Abstract

Inferring brain functional connectivity from functional magnetic resonance imaging (fMRI) data extends our understanding of systems-level functional organization of the brain. Functional connectivity can be assessed at the individual voxel or Region of Interest (ROI) level, with pros and cons of each approach. This thesis focuses on addressing fundamental problems associated with ROI-based brain functional connectivity inference, including regional signal representation, brain functional connectivity modelling and brain functional connectivity analysis.

Functional connectivity involving brainstem ROIs has been rarely studied. We propose a novel framework for brainstem-cortical functional connectivity modelling where the regional signal of brainstem nuclei is estimated by Partial Least Squares and connections between brainstem nuclei and other cortical/subcortical brain regions are reliably estimated by partial correlation. We then apply the proposed framework to assess functional connectivity of one particular brainstem nucleus – the pedunculopontine nucleus (PPN), which is important for ambulation, and is affected in diseases putting people at risk for falls (e.g., Parkinson’s Disease).

A key issue for ROI-based brain functional connectivity assessment is how to summarize the information contained in the voxels of a given ROI. Currently, the signals from the same ROI voxels are simply averaged, neglecting any inhomogeneity in each ROI and assuming that the same voxels will interact with different ROIs in a similar manner. In this thesis, we develop a novel method of representing ROI activity and estimating brain functional connectivity that takes the regionally-specific nature of brain activity, the spatial location of concentrated activity, and activity in other ROIs into account.

Finally, to facilitate the interpretation of the estimated brain functional connectivity networks, we propose the use of dynamic graph theoretical measures (e.g., the newly introduced graph spectral metric, Fiedler value) as potential MRI-related biomarkers.

The proposed methods were applied to real fMRI datasets, with a primary focus on Parkinson’s disease. The proposed methods demonstrated enhanced robustness of brain functional connection estimation, with potential use in disease assessment and treatment evaluation. More
broadly, this thesis suggests that brain functional connectivity offers a promising avenue for non-invasive and quantitative assessment of neurological diseases.
Lay Summary

Functional magnetic resonance imaging (fMRI) is a way to non-invasively assess the brain in action. Instead of determining which areas of the brain activate, another way of assessing the brain is to look at brain areas that appear to work together. We specifically looked at how we could improve our ways of determining how brain Regions of Interest (ROIs) interact with one another. One improvement was to examine the case when an ROI was part of the brainstem – a small vital structure at the base of the brain. Another improvement was how to summarize the activity within an ROI, even when a single ROI may encompass different clumps of activity. We also examined how connectivity between ROIs change over time. While our emphasis was on Parkinson’s Disease, our approaches can be used to assist in evaluation of a number of brain diseases, and in assessing normal brain functioning.
Preface

The work in this thesis is conducted by the candidate, under the supervision of Dr. Z. Jane Wang and Dr. Martin J. McKeown. This thesis is based on a collection of manuscripts that have been published in international journals and conferences or submitted for publications.

Chapter 2 is based on the following manuscripts:


The author was responsible for data analysis and writing the manuscript. The work was conducted with the guidance and editorial input from Dr. Z. Jane Wang and Dr. Martin J. McKeown. The experimental fMRI data was provided by Dr. Martin J. McKeown (UBC Clinical Research Ethics Board: H09-02016). Dr. Soojin Lee, Laura J. Kim, and Diana Kim conducted the experiment and data acquisition, and Saurabh Garg performed the preprocessing of fMRI data. Dr. Fang Ba and Dr. Soojin Lee contributed to neurological interpretations of the results and editorial input on the manuscript. Dr. Aiping Liu provided valuable feedback on the data analysis and editorial input on the manuscript.

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The author was responsible for the development of the proposed algorithm, performing simulations and real fMRI application, and writing the manuscript. The work was conducted with the guidance and editorial input from Dr. Z. Jane Wang and Dr. Martin J. McKeown. Yuheng Wang contributed to data organization and part of the implementation of the experiment. Dr. Aiping Liu contributed to the formulation of the problem, evaluation of the methods, and providing editorial input and valuable feedback.

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  The author contributed to part of the data analysis and provided editorial input on the manuscript. Yuheng Wang was responsible for formulating the problem, implementing the proposed method, analyzing the results and writing the manuscript. Daniel C. Louie collected the experimental data and provided editorial input on the manuscript. Dr. Z. Jane Wang, Dr. Tim K. Lee and Dr. Harvey Lui provided valuable feedback and helped edit the manuscript.


  The author contributed to part of the data analysis and provided editorial input on the manuscript. Yuheng Wang was responsible for formulating the problem, implementing the proposed method, analyzing the results and writing the manuscript. Daniel C. Louie collected the experimental data and provided editorial input on the manuscript. Dr. Z. Jane Wang, Dr. Harvey Lui and Dr. Tim K. Lee provided valuable feedback and helped edit the manuscript.

- Aiping Liu, Soojin Lee, Jiayue Cai, Taomian Mi, Saurabh Garg, Laura Kim, Maria Zhu, Xun Chen, Z. Jane Wang and Martin J. McKeown, “Galvanic Vestibular Stimulation
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The author contributed to part of the data analysis. Dr. Aiping Liu was responsible for the problem formulation, data analysis and writing the manuscript. Dr. Soojin Lee, Taomian Mi, Laura Kim, Maria Zhu conducted the experiment and collected the data. Dr. Soojin Lee contributed to the medical interpretation and provided editorial input. Saurabh Garg performed the preprocessing of the data. Dr. Martin J. McKeown, Dr. Z. Jane Wang and Dr. Xun Chen provided valuable feedback and helped edit the manuscript.
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# Glossary

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<th>Full Form</th>
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<td>AD</td>
<td>Alzheimer disease</td>
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<tr>
<td>AR</td>
<td>Autoregressive</td>
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<tr>
<td>BOLD</td>
<td>Blood oxygen level dependent</td>
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<td>BSS</td>
<td>Blind source separation</td>
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<tr>
<td>CCA</td>
<td>Canonical correlation analysis</td>
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<tr>
<td>CSF</td>
<td>Cerebrospinal fluid</td>
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<tr>
<td>DAG</td>
<td>Directed acyclic graph</td>
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<tr>
<td>DBS</td>
<td>Deep brain stimulation</td>
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<td>DCA</td>
<td>Discriminant correlation analysis</td>
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<td>DCM</td>
<td>Dynamic causal model</td>
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<tr>
<td>DEC</td>
<td>Deep embedded clustering</td>
</tr>
<tr>
<td>DEPICT</td>
<td>Deep embedded regularized clustering</td>
</tr>
<tr>
<td>DTI</td>
<td>Diffusion Tensor Imaging</td>
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<tr>
<td>EEG</td>
<td>Electroencephalogram</td>
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<tr>
<td>FDR</td>
<td>False discovery rate</td>
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<tr>
<td>fMRI</td>
<td>Functional magnetic resonance imaging</td>
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<tr>
<td>FOG</td>
<td>Freezing of gait</td>
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<tr>
<td>GLM</td>
<td>General Linear Model</td>
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<tr>
<td>GPi</td>
<td>Globus pallidus internus</td>
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<td>GVS</td>
<td>Galvanic vestibular stimulation</td>
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HCP Human connectome project
HC Healthy control
ICA Independent component analysis
KKT Karush-Kuhn-Tucker
LA-cCCA Local activity constrained canonical correlation analysis
LASSO Least absolute shrinkage and selection operator
LDA Linear discriminant analysis
MAR Multivariate autoregressive
MCCA Multiset canonical correlation analysis
MCI Mild cognitive impairment
MEG Magnetoencephalogram
MLC Mesencephalic locomotor center
PCA Principal component analysis
PD Parkinson’s disease
PET Positron emission tomography
PI Postural instability
PIGD Postural instability gait difficulty
PLS Partial least squares
PPN Pedunculopontine nucleus
RBF Radial base function
RMSE Root of mean square error
ROI Region of interest
SEM Structural equation model
SMA Supplemental motor
SNC Substantia nigra pars compacta
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>SNr</td>
<td>Substantia nigra pars reticulata</td>
</tr>
<tr>
<td>SNR</td>
<td>Signal to noise ratio</td>
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<tr>
<td>STN</td>
<td>Subthalamic nucleus</td>
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<tr>
<td>SVM</td>
<td>Support vector machine</td>
</tr>
<tr>
<td>TD</td>
<td>Tremor dominant</td>
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<tr>
<td>UPDRS</td>
<td>Unified Parkinson’s disease rating</td>
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My doctoral study has been a challenging, memorable, and happy experience, with the generous support of many people. I would like to take this opportunity to thank all of them for being with me throughout this journey.

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Chapter 1

Introduction

The human brain, then, is the most complicated organization of matter that we know. – Isaac Asimov

Our brain is one of the most important and intricate parts of a human body, which controls our actions, thoughts, feelings and memory. It has been a long-standing challenge for neuroscientists to understand the underlying mechanism of the brain. A tremendous number of interacting neural elements consist of a complicated brain network. The mapping of brain networks by conducting a connectivity analysis has thus been a rapidly moving field, advancing the understanding of the organization of the brain.

Brain connectivity can be delineated as structural or functional. Structural connectivity denotes anatomical links (e.g., synapses or fiber pathways) of neuronal elements (neurons or brain regions). It measures the physical presence of axonal projection in the brain, which can be detected by diffusion tensor imaging. Such anatomical architecture supports the emergence of physiological activity, giving rise to the concept of functional connectivity. Functional connectivity models the statistical dependence between spatially distributed neuronal elements, which is considered to provide the basis for information processing and mental representation. While structural connectivity provides the anatomical basis for functional connectivity, the existence of functional connectivity is not only limited to direct structural connections, but also can be derived from indirect anatomical connections via mediating brain regions. In this thesis, we focus on functional connectivity to elucidate the functionally interregional coordination of the brain.

Brain functional connectivity provides insights into the organizations of the brain in both healthy and diseased states. Neurological diseases, such as Alzheimer disease (AD), Parkinson’s disease (PD), schizophrenia, affect a large population worldwide. Their relatively high prevalence and serious consequences have a significant impact on global health. Of particular importance, neurological disorders can be characterized as dysconnection syndromes [163]. The
abnormal brain functional connectivity patterns could serve as potential biomarkers for neurological diseases, assisting in the clinical diagnosis of the diseases. In addition, brain functional connectivity offers a promising way for the evaluation of therapeutic intervention. Therefore, the aim of this thesis is to develop novel methods to assess brain functional connectivity and explore appropriate ways to quantitatively interpret/analyze the estimated brain functional connectivity networks, which ultimately leads to a better understanding of functional connectivity changes induced by neurological diseases and/or clinical interventions.

In this chapter, we provide a comprehensive introduction on brain functional connectivity and outline the objectives of this thesis. We first start with an overview on functional connectivity using fMRI data in Section 1.1 followed by Section 1.2, 1.3 and 1.4 where we review current popular methods across the general pipeline of brain functional connectivity study, that is, regional signal representation, brain functional connectivity modelling and brain functional connectivity analysis respectively. In Section 1.5, we then present the research objectives of this thesis. Finally, we describe the thesis outline in Section 1.6.

1.1 An Overview on Functional Connectivity

There are diverse modalities for studying brain functions, including Electroencephalogram (EEG), Magnetoencephalogram (MEG), Positron Emission Tomography (PET) and fMRI. EEG measures neural activity by recording electrical signals through electrodes placed on the scalp, and MEG measures brain activity by monitoring magnetic fields generated by electrical activity through sensitive magnetometers. These two neuroimaging techniques have advantages in studying the temporal dynamics of neural activity because of the high temporal resolution at the level of milliseconds. Nevertheless, they have a low spatial resolution with the level of centimeters and exhibit difficulties in localizing the underlying brain activity. PET is an imaging technique that measures brain metabolism and radioactivity by using a radioactive drug (tracer) to reflect the neural activity. It has a particular advantage in studying brain neurophysiology and neurochemistry, such as monitoring the activity at dopamine receptors which is very useful in the study of PD. Nevertheless, the temporal resolution of PET is very low from tens of seconds to minutes, and the injection of radioactive tracer is required in a PET scan, which becomes limitations of this imaging technique.

Among various neuroimaging techniques, fMRI has become prevalent and widely adopted,
in large part due to a relatively high spatial resolution and the non-invasiveness. It measures the relative changes in the blood oxygen level-dependent (BOLD) signal induced by neural activity. The BOLD signal indirectly measures brain activity via the effects on blood oxygenation and blood flow, affecting magnetic properties and MRI contrast in turn. Figure 1.1 shows an example of fMRI scan and the resulting fMRI signals.

![Figure 1.1: (a) A typical fMRI scan and (b) the resulting fMRI signals. Image courtesy of [173].](image)

Conventional fMRI analysis has focused on determining brain areas activated during the conduction of certain tasks or under specific stimuli. In order to detect the effects of interest, it is necessary to use contrasts and repetitions in the fMRI experiment. This is usually done by utilizing a block design, where subjects are asked to alternate between the task of interest and some control task such as at rest and repeat the tasks as often as possible. Figure 1.2(a) shows an example of a block design involving alternations between finger-tapping and rest.

To locate brain activation in response to a certain task, statistical analysis is typically performed using the univariate approach, i.e., by constructing a separate model at each voxel. The most commonly used method is the General Linear Model (GLM) [67], which considers the fMRI signals as a linear combination of model functions and noise. The GLM is formulated as

\[ Y = X\beta + \epsilon \]  

(1.1)

where \( Y \) is a vector representing the fMRI time courses from a single voxel, \( X \) is the design matrix reflecting the experimental design factors included in the model, \( \beta \) is a vector corresponding to the model parameters and \( \epsilon \) is the error term.

After GLM is separately fitted at each voxel, a statistical test is performed on the estimated model parameters to examine if each voxel is activated significantly in response to the task.
Figure 1.2: An example of brain activation study. (a) Block design in a fMRI experiment. Subjects perform the experiment of alternating blocks of finger-tapping and rest. (b) Statistical parameter map for one subject. The colored regions indicate significant brain activation in response to a certain task (e.g., a finger-tapping task). Image adaption from [185].

This results in a statistical parameter map across all voxels, where voxels with task-related significant activation are colored according to their significance levels (see Figure 1.2(b) for an example).

Rather than determining the isolated brain areas activated under experimental conditions as mentioned above, recent fMRI studies have witnessed a change of focus from functional specialization to functional integration. The study of functional integration aims to examine how different brain regions connect, interact and communicate with each other, giving rise to the concept of “brain functional connectivity”. Brain functional connectivity provides a macroscopic view of functional coordination of the interacting brain regions. Since many neurological diseases can be delineated as dysconnectivity syndromes, brain functional connectivity study offers promising ways for developing MRI-related biomarkers and understanding abnormal functional integration of diseased brains.

With the emergence of brain functional connectivity, a particular type of paradigm, the so-called “resting-state” becomes prevalent in fMRI study. Unlike task-related brain activation studies, resting-state fMRI, wherein the individuals are free of any task and required to relax without thinking of anything, is well-suited to brain functional connectivity analyses, as the
intrinsic co-activation patterns of the brain are reflected. The studies of resting-state brain functional connectivity revealed that the brain is not idle during rest, but rather exhibits substantial spontaneous neuronal activity.

Inferring brain functional connectivity from fMRI data can take place at voxel or Region of Interest (ROI) level. In voxel-based models, there are usually a large amount of voxels involved in the analysis, making such models less attractive in practice particularly since some models (e.g. Bayes network models) become impractical with very high-dimensional data. Second, statistical power is dramatically reduced due to the need to correct for multiple comparisons. Another critical issue is dealing with the fact that people’s brains are of different size and shape. To perform a group study using a voxel-based analysis, all brain images must be spatially transformed to a common template, possibly inducing registration errors, with unknown effects on downstream analyses. In contrast, with an ROI-based analysis, the ROIs can be defined in each subject’s native space, without the need for a common registration process. Therefore, ROI-based models have been frequently adopted for analyses in fMRI studies. Figure 1.3 shows the general pipeline for the ROI-based brain functional connectivity study.

Figure 1.3: An overview on the general pipeline of the ROI-based brain functional connectivity study.
1.2 Regional Signal Representation

A key issue for assessing the ROI-based brain functional connectivity is how to represent the information in a given ROI. A common practice is to simply take the average signal from same-ROI voxels for regional signal representation. The simpleness and straightforwardness of the average signal method make it very popular and widely adopted by functional connectivity studies. One drawback of this method lies in the possible functional inhomogeneity of the ROI. Since one ROI may actually encompass several functional sub-regions [88, 115], the use of the average signal ignores the intrinsic data structure and is prone to introduce biases, thus less optimally reflecting the on-going activity in the ROI [150, 206].

Another way to represent ROI activity is to select a “representative” voxel for the ROI signal representation. This representative voxel can be chosen based on a pre-defined seed voxel [175] [186], or fMRI activation [176]. For the activation-based selection, the peak activated voxel can be selected as the representative voxel and its time signal can be used as the ROI representative signal. Considering the fact that a single voxel may not be fully representative, an extensive way is to select a cluster of voxels around the peak voxel and take the average time signal of selected voxels as the ROI representative signal. Several strategies can be used to choose the surrounding voxels, such as clusters that contain the peak voxel, a sphere or a few top contiguous voxels around the peak voxel.

Alternatively, Principal Component Analysis (PCA) can be performed on time series of all voxels in a ROI and the first principal component can be used as the ROI representation signal. In [205], the combination of PCA and regression model was developed to assess brain functional connectivity from fMRI data. Another study used PCA in conjunction with Granger causality to examine causal influences between distinct brain regions [206] [207]. This provides an alternative method for ROI signal representation in addition to the average signal method. However, the PCA method may also tend to be sensitive to functional inhomogeneity and exhibit poor reliability [181].

To date, there have been limited studies investigating related approaches on regional signal representation, compared to the relatively large number of studies developing novel brain functional connectivity modelling methods (on top of the estimated ROI signals). However, this is a general research problem worthy of careful investigation, since different ROI signal representation strategies may significantly impact the subsequent results inferred from fMRI.
data [176]. Extensive work will be necessary to develop appropriate regional signal representation approaches that take into account intrinsic data structure of the ROIs for a reliable fMRI analysis.

1.3 Brain Functional Connectivity Modelling

Human brain can be considered as a complicated network consisted of a large number of different brain regions that interact with each other. Brain connectivity, particularly in this thesis, functional connectivity, studies such interaction relationship by examining the statistical association between distinct brain regions. Brain functional connectivity modelling is often proceeded by specifying a set of “functional nodes” and then analyzing functional connectivity between these nodes. In ROI-based models, the nodes usually correspond to spatial ROIs identified based on anatomical brain atlas. Alternatively, the spatially independent components (accompanying by their associated time courses) obtained from independent component analysis (ICA) can also be used to define the nodes [4, 146, 195]. A variety of approaches have been developed for estimating brain functional connectivity between the ROIs, ranging from pairwise measures to global network models, from linear to non-linear measures, and from directional to bidirectional models.

The most straightforward one is correlation coefficient. The more correlated the time courses are between two brain regions, the more possible it is that there is a functional connection between them. Therefore, we can evaluate the brain functional connectivity by computing the correlation coefficients between any pair of brain regions and by setting up a threshold, with those pairs of brain regions whose correlation coefficients are larger than the threshold being considered associated with each other. However, correlation does not give necessary information regarding whether or not the functional connectivity between a pair of brain regions is direct. In this case, another simple yet effective method, partial correlation [53, 107], is a more appropriate way to infer the direct brain functional connectivity, since it works by estimating the correlation between two time courses with the effect from other variables being removed. In addition to the correlation method, mutual information [36, 204] is another widely used estimation method which quantifies the shared information between two time courses and reflects linear as well as
nonlinear dependence relationships (Equation 1.2).

\[ I(X, Y) = \sum_{x \in X} \sum_{y \in Y} p(x, y) \log \frac{p(x, y)}{p(x)p(y)} \] (1.2)

where \( X \) and \( Y \) are two random variables, \( p(x, y) \) is the joint probability of \( X \) and \( Y \), and \( p(x) \), \( p(y) \) are two marginal probabilities of \( X \) and \( Y \) respectively.

Correlation, partial correlation and mutual information are popular estimation approaches, however, they cannot solve the problem of the direction of the functional connection. For the estimation of the direction, one of the common approaches is Granger causality [40, 140] which is a lag-based measurement. It defines causality based on the statistical interpretation where one variable \( X \) is considered to cause another variable \( Y \) if \( X \) can at least partially contribute to the prediction of \( Y \). Another available method is Patel’s conditional dependence measure [129, 154] which estimates the causality by looking at the imbalance of conditional probability between two variables.

Different from the aforementioned pairwise brain functional connectivity estimation methods, some multivariate regression models, such as structural equation model (SEM) [21, 153], multivariate autoregressive (MAR) model [77, 144] and dynamic causal model (DCM) [66, 171], have also been widely used for brain functional connectivity studies. While SEM aims to estimate the instantaneous interaction between brain regions, MAR characterizes inter-regional relationships utilizing lag-based models which incorporates temporal effects one brain region has on another. The MAR method models one sample of an N-dimensional time courses as a linear combination of its previous ones, as formulated in Equation 1.3. Different from SEM and MAR, DCM deals with the nonlinear and dynamic activities between brain regions, and the neuronal activities are modeled as hidden variables.

\[ x_t = \sum_{p=1}^{P} A_p x_{t-p} + \epsilon_p \] (1.3)

where \( x_t \) is an N-dimensional vector representing the fMRI signal of N ROIs at the \( t \)th time point, \( p \) is the order of the model with the maximum value of \( P \), \( A_p \) is an \( N \times N \) matrix of coefficients (weights) at the time lag of \( p \), and \( \epsilon_p \) is the error term.

The multivariate regression models are statistically rigorous and relatively flexible methods with many available algorithms being developed. However, one disadvantage of these models
concerns their computational feasibility. When considering the computational cost, regularized inverse covariance is an effective way to assess the brain functional connectivity. The inverse covariance matrix (precision matrix) has been used to infer brain functional connectivity, encoding conditional independence relationships between brain regions. Under the assumed sparse nature of brain functional connectivity networks, a regularization strategy, such as the LASSO method \(^1\) can be applied to impose a sparsity constraint on the inverse covariance matrix, leading to a sparsity structure on the coefficients \([31, 64]\). A sparse estimate of the inverse covariance matrix can be obtained by minimizing the penalized negative log likelihood

\[
\hat{\Theta} = \arg \min \{ \text{tr}(S\Theta) - \log |\Theta| + \lambda \|\Theta\|_1 \} \tag{1.4}
\]

where \(\Theta\) is the inverse covariance matrix, \(S\) is the sample covariance matrix, \(\|\Theta\|_1\) is the element-wise L1-norm of \(\Theta\), and \(\lambda\) is the penalty parameter controlling the sparsity of the network.

Graphical models have been employed to estimate brain functional connectivity and attracted increasing attention in the field of brain functional connectivity network modelling. Bayesian network \([119, 188]\) is a typical graphical model which is based on conditional independence relationships among the variables. For example, PC algorithm ("Peter and Clark"; \([114]\)) is one of the popular Bayesian network modelling methods. It searches for causal graphs under the constraint of directed acyclic graph (DAG), first constructing the structure of the graph based on conditional independence and then determining directions with a specific set of rules. An extension of PC algorithm was also proposed by incorporating a false discovery rate (FDR) control \([97]\).

Overall, brain functional connectivity modelling is a rapidly moving research area, attracting a large number of researchers to make diverse contributions. Based on different assumptions, a variety of novel approaches have been proposed to address certain aspects of brain functional connectivity modelling, all having their own advantages and limitations. In real applications, different methods have been employed according to the specific demands in certain scenarios, resulting in a collection of significant findings that provide insights into disease mechanism in terms of brain functional connectivity. Recent fMRI studies have witnessed a huge growth in the area of brain functional connectivity; over the coming years, brain functional connectivity

\(^1\)LASSO: Least Absolute Shrinkage and Selection Operator, a regularization method for linear regression which minimizes the objective function with a bound on the sum of the absolute values of the coefficients.
will remain a vital tool and receive further growth regarding both technical and modelling challenges, and its applications.

1.3.1 Brainstem Functional Connectivity Modelling

Brainstem is extremely important in the neural system, which is located at the posterior part of the brain, as shown in Figure 1.4. Many neurological diseases can involve in functional disruptions of the brainstem, such as PD, AD and multiple sclerosis. Even small lesions in the brainstem can have profound effects and result in serious neurological deficits. There are various nuclei in the brainstem, which are important components of almost every functional neural system, controlling for many body functions such as motor, sensory, circulation, respiration and mood. It has been suggested by some experts that a complete dysfunction of the brainstem is equal to the death of the brain.

![Figure 1.4: An illustration on anatomical structures of the brain. Image adaption from [190].](image)

Exploring functional connectivity of the brainstem not only facilitates a comprehensive understanding of brain functions, but also contributes to unravelling the pathogenesis of many brain disorders and the development of potential treatment strategies. In clinics, brainstem remains a major target for many neurological disorders. For example, in PD, gait impairment is associated with increased fall risk, which remains a major source of morbidity in patients. The pedunculopontine nucleus (PPN) is one of the brainstem nuclei, which importantly involves in the control of gait due to its close interconnections with cortical motor areas. Inspired by its vital function, a novel treatment, deep brain stimulation (DBS), has been developed targeting at the PPN for ameliorating gait freezing in PD.
Despite the indisputable importance of the brainstem, it is frequently neglected by neuroimaging communities in the studies of brain functions and dysfunctions. To date, the vast majority of fMRI studies have been focused on the functional connectivity between cortical and subcortical brain regions, however, the functional connectivity of the brainstem is poorly understood. Several reasons that hindered the study of brainstem functional connectivity include: (1) Compared to other cortical/subcortical brain regions, brainstem nuclei are always very small with an average diameter level of only a few millimeters, which makes it difficult to localize their anatomical structures. (2) Due to its peculiar anatomical locations, fMRI signals from the brainstem suffer from strong physiological noise including respiration and pulsation. (3) Different brainstem nuclei with distinct functions can be located very close to each other, leading to difficulties in distinguishing these structures.

Although brainstem is a tough structure which exerts difficulties on related research, the recent few years have witnessed some significant advances in brainstem fMRI studies. Some pioneering studies have successfully examined the activity of single brainstem nuclei [52, 57, 174]. Consecutively, Baissner et al. investigated for the first time the inter-nuclear and nucleo-cortical connectivity, wherein they identified intrinsic brainstem networks and inferred the brainstem-cortical functional connectivity using fMRI data [16]. More recently, Bär et al. investigated topological characteristics of brainstem nuclei using graph theory. These studies advanced the fundamental understanding of human brainstem functions [12]. In addition, other studies investigated the mechanism of functional brainstem disruptions in neurological diseases, suggesting altered brainstem functional connectivity patterns in patients [58, 156]. Such studies demonstrated contributions of brainstem degeneration to the pathophysiology of brain diseases and provided insights into potential biomarkers and treatment strategies of neurological disorders.

With regard to the methods, technically speaking, all general brain functional connectivity modelling methods can be used to estimate the brainstem functional connectivity. For example, in voxel-level analysis, seed-based analysis was utilized to estimate functional connectivity of several brainstem nuclei in [156]; another study applied ICA to detect functional brainstem networks from the whole brainstem BOLD signals [16]. Other popular approaches, such as (partial) correlation and SEM were also utilized to assess brainstem functional connectivity [12, 83, 167].

It should be noted that because of the anatomical peculiarity one needs to be particularly careful when inferring brainstem functional connectivity. Due to the structural differ-
ences between cortical/subcortical regions and brainstem, many procedures operated on cortical/subcortical regions may be sub-optimal for brainstem structures. For example, since brainstem nuclei are always quite small, the error can be larger than the average size of the studied structures during the whole brain registration, leading to less precise results. Therefore, it would be desirable to use appropriate procedures specially tailored for brainstem structures. In [12], a separate fMRI preprocessing steps were carried out on the brainstem to avoid potential problems. In addition, special care needs to be taken to reduce physiological noise in brainstem structures. This can be done by recording physiological signals during fMRI scans and then performing temporal noise regression [26]. Alternatively, Baissner et al. developed a novel masked ICA method to suppress physiological noise of the brainstem [16]. This method restricted ICA analysis to an anatomical brainstem mask based on the observation that the major physiological noise came from the vicinity instead of the inside of the brainstem. Another issue concerns the BOLD signal representation of brainstem nuclei. Despite the small size of brainstem nuclei, they tend to have a relatively large functional heterogeneity. Hence, the commonly used average time courses may not optimally represent brainstem ROI signals. Appropriate ROI signal representation methods would be in demand for brainstem fMRI studies.

1.3.2 Time-varying Functional Connectivity Modelling

It is of great interest in assessing the brain functional connectivity, advancing our understanding of functional brain organizations. However, most brain functional connectivity studies have been conducted with the assumption that the interactions between distinct brain regions are invariant, and thus providing static descriptions of functional connectivity. Until recently, it has been proposed that investigating temporal variations in functional connectivity may offer greater insights into fundamental brain network properties [82]. This encourages the emergence of the modelling of dynamic brain functional connectivity.

Sliding window analysis is a common method for examining the dynamics in brain functional connectivity. This approach works by identifying a time window of fixed length and shifting this time window by a certain number of data points. In principle, any functional connectivity metric that can be applied to the static-assuming investigations, such as correlation, can be used to assess the time dependent functional connectivity at each time window in the sliding window analysis. Recently, regularized precision matrix [189] and multiplication of temporal derivatives [157] have been introduced for the within-window functional connectivity estimation. However,
one issue concerning the sliding window approach is choosing the window size. If the window size is too small, it will not allow for the robust estimation of functional connectivity; otherwise, if the window size is too large, it will not detect the potential interesting transients. Also, the common choice of window shape, rectangular window which assigns the same weights to the time points inside the window, may increase the sensitivity to the noise. Alternatively, tapered window has been employed in some recent studies [13, 43].

To circumvent the issue associated with the window choice, one may consider using time-frequency analysis [37, 198] which can be implemented with the wavelet transform coherence. With this approach, there is no need to apply a fixed window size. Instead, the effective window is adapted according to the intrinsic time-scale of the frequencies in original signals. Time-frequency analysis allows the temporal exploration of functional connectivity across a range of frequencies, resulting in a rich time-frequency map for each pair of ROIs. One drawback of this method concerns the large number of information resulted from the analysis, which induces additional steps to handle the growth of outputs.

Another approach that avoids the arbitrary choice of window is to use a data-driven method to determine time change points in brain functional connectivity, which is called dynamic connectivity regression (DCR) [49]. This method performed a recursively temporal partition using a binary search tree structure and estimated interaction relationships between ROIs using graphical Lasso. It enables both the detection of change points and the estimation of functional connectivity within each temporal interval. However, the computational cost of DCR method can be extremely high with a large number of ROIs. To deal with high-dimensional data, an adjusted version, called dynamic connectivity detection (DCD) [197], can be employed to infer the time-varying functional connectivity.

Efforts towards the explicit temporal modelling, i.e., including the influence of time on brain functional connectivity in the computational model, have also been dedicated for studying dynamic functional connectivity. One example is the modelling of temporal smoothness, that is, temporally adjoining brain functional connectivity networks have similar network structures and connection strengths. Such studies include fused multiple graphical lasso (FMGL) [189], sticky weighted time-varying model (SWTV) [99] and time-varying graphical lasso (TVGL) [29]. In these studies, the temporal smoothness was achieved by imposing a fused penalty which encouraged the adjoining brain functional connectivity networks to have similar patterns. Another attempt is to apply hidden Markov model (HMM) to model dynamic brain functional
connectivity \cite{56,169}, wherein the brain is assumed to stay at one particular state corresponding to every time point.

Since the initial findings on dynamic behavior of functional connectivity, increasing attention has been paid to investigate time-varying functional connectivity. Capturing temporal changes on functional connectivity enables the exploration on the full extent of brain activity and expands our understanding of the dynamic evolution of functional networks. Although many methodological variants have been proposed, studies on examining the utility of brain dynamics for assessing brain disease states are still in its infancy. Further work on the interpretation of dynamic brain functional connectivity networks and its underlying relationship with neurological disorders will be in demand, providing new potential biomarkers on MRI assessment of brain diseases.

1.4 Brain Functional Connectivity Analysis

In the last section, we focused on the examination of brain functional connections between distinct brain regions. By extension, another problem of interest is how to extract useful information from the inferred brain functional connectivity network. Such process will help answer many exciting questions such as what the underlying organizations of brain functional connections are, how well our brain can process the information and which brain regions may act as specialized roles in this information processing system.

One popular way to extract meaningful information from functional connectivity networks is to utilize graph theoretical analysis, which characterizes the architecture and information flow of brain networks. One of the popular graph theoretical studies using fMRI data is related to small-worldness of brain networks, which displays both short path lengths and high clustering levels \cite{28}. There have been studies showing the small-worldness in human brain networks \cite{15}, indicating that our brain is an efficient information processing system. Also, topological properties of brain networks can be quantitatively described by many other graph measures such as clustering coefficient, modularity, global efficiency, characteristic path length, centrality and node degree \cite{28}. These topological properties help provide a comprehensive understanding of brain network organization, and assist in the assessment of disease states. In literature, altered graph theoretical properties have been found in different neurological diseases, such as AD, PD and schizophrenia \cite{10,14,170}, suggesting the abnormal functional organization of...
diseased brain networks.

With the prevalence of dynamic brain functional connectivity studies, dynamic graph theoretical analysis has been explored as well, examining the temporal changes in topological properties of brain networks. By applying dynamic graph theoretical analysis, graph measures are computed on each temporal brain network, yielding a series of graph measures over time. It should be mentioned that temporal models (e.g., the smoothness between subsequent time points) can also be used in the level of graph measures [118]. Recent studies have shown that various graph measures exhibited temporal fluctuations, suggesting functional reorganization of brain networks over time [203].

In particular, further analysis can be required for dynamic brain functional connectivity networks to get potentially useful insights from the rich temporal and spatial information. For example, clustering methods can be applied to the correlation matrices calculated from sliding window analysis to extract reproducible, transient functional connectivity patterns, which can be termed as “connectivity states”. K-means clustering [4] and hierarchical clustering [201] have been employed in such a manner to extract connectivity states. Alternatively, modularity [202], temporal ICA [199], PCA [96], HMM [125] approaches have also been introduced to describe dynamic functional connectivity states.

Extracting useful information from the inferred brain networks prompts the meaningful interpretation of functional connectivity. Such analysis advances our understanding of underlying working mechanism of both the healthy and diseased brains. Further work will be of interest to discover effective network features for the assessment of diverse neurological diseases.

1.5 Research Objectives

The study of brain functional connectivity provides deep insights into the large-scale brain functional coordination. It not only helps with the understanding of the fundamental mechanism of brain functioning at the normal state, but also reveals the disrupted brain interaction patterns at the diseased state. While inferring brain functional connectivity can take place at voxel or ROI level, this thesis focuses on the latter and aims to develop novel methods to address fundamental problems associated with ROI-based brain functional connectivity study, including regional signal representation, brain functional connectivity modelling, and brain functional connectivity analysis. Additionally, brain ROIs can be broadly categorized into cortical/subcortical ROIs
and brainstem structures, we aim to study both of them in this thesis.

Motivated by the particular applications in our study, we are interested in addressing the following concerns and challenges. First, the brainstem is an extremely important part of the brain involving in almost every function of human bodies. Inferring brainstem functional connectivity can make a profound contribution to understanding the pathophysiology of brain diseases and developing treatment strategies. However, relatively few studies have been conducted to model functional connectivity of the brainstem, in large part due to its anatomical peculiarity. It is a challenging problem to get reliable fMRI signals from the complicated brainstem structures and represent their regional signals in a reasonable manner.

Second, representing the signal in a given ROI is an important issue for assessing the ROI-based brain functional connectivity. With most current approaches, the signals from same-ROI voxels are simply averaged, neglecting any inhomogeneity in each ROI and thus less optimally reflecting ongoing activity in the ROI. Therefore, improved brain functional connectivity modelling methods that incorporate intrinsic data structure for regional signal representation are required to guide the fMRI analysis.

Finally, interpretation of the inferred brain functional connectivity networks is also challenging because of the spatial complexity. After the estimation of brain functional connectivity networks, extracting the useful information from the complex network, particularly in the dynamic setting which contains rich spatiotemporal information, is anything but easy. Seeking appropriate summary measures is required to facilitate the interpretation and assist in the assessment of brain disease states.

The objective of this thesis is to address the aforementioned challenges present in the general pipeline of ROI-based brain functional connectivity study, including regional signal representation, brain functional connectivity modelling, and brain functional connectivity analysis. Specifically, the main contributions of the thesis are summarized as follows:

- Propose a brainstem-cortical functional connectivity modelling framework which incorporates a novel brainstem regional signal representation method and special care in the preprocessing. The proposed framework was applied to assess functional connectivity of one particular brainstem nucleus – pedunculopontine nucleus, and perform treatment evaluations of two kinds of therapeutic interventions in PD.

- Develop a novel method of representing ROI activity and estimating brain functional
connectivity that takes the regionally-specific nature of brain activity, the spatial location of concentrated activity, and activity in other ROIs into account.

- Propose the use of dynamic graph theoretical measures to extract useful information from brain functional connectivity networks as potential MRI-related disease biomarkers. In particular, a novel graph spectral metric, Fiedler value, is introduced for studying the dynamics of brain functional connectivity.

Figure 1.6 illustrates challenges and objectives of the thesis.

1.6 Thesis Outline

The rest of the thesis is outlined as follows:

In Chapter 2 and Chapter 3, we propose a novel framework for brainstem-cortical functional connectivity modelling where the regional signal of brainstem nuclei is represented by using partial least squares approach and the connections between brainstem nuclei and other cortical/subcortical brain regions are estimated by partial correlation. Additionally, considering the anatomical peculiarity of the brainstem, in our proposed framework, special care is taken in the preprocessing wherein a separate brainstem motion correction is performed. We apply the proposed framework to assess functional connectivity of one particular brainstem nucleus – pedunculopontine nucleus. Specifically, we investigate the effect of Galvanic Vestibular Stimulation (GVS) and walking exercise on the PPN functional connectivity in PD respectively.

In Chapter 4, we propose a novel method for simultaneous regional signal representation and brain functional connectivity estimation. The proposed method detects regional-specific nature of brain activity via density clustering and incorporates such intrinsic structure into ROI signal representation and brain functional connectivity estimation via constrained canonical correlation analysis. We evaluate the proposed method on both simulated and real fMRI data, resulting in higher accuracy of brain functional connectivity estimation and/or a more reproducible connectivity pattern.

In Chapter 5, to facilitate the interpretation of the estimated brain functional connectivity networks, we leverage graph measures to extract useful information from dynamic brain functional connectivity networks. Dynamic graph measures and the potentials of such dynamics

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1 Ambulosono: A home-based music walking program.
2 CCA: Canonical Correlation Analysis.
as MRI-related biomarkers of PD are investigated. In particular, we propose a novel graph spectral measure to characterize global integration of brain functional connectivity networks in PD and evaluate its important role in the disease assessment.

Finally, Chapter 6 presents a summary of the thesis contributions and discusses future work.
Figure 1.5: An overview of the challenges and objectives of this thesis.2
Chapter 2

PLS-based Regional Signal Representation for PPN Functional Connectivity Estimation in PD: fMRI Effect of GVS

Anatomically, the brain can be broadly divided into two categories: cortical/subcortical regions and brainstem structures. While functional connectivity between cortical/subcortical regions is commonly studied, functional connectivity between brainstem structures and cortical/subcortical regions is rarely investigated. In this chapter, we propose a novel framework to assess brainstem-cortical functional connectivity, wherein a separate brainstem motion correction is performed to cope with the anatomical peculiarity of the brainstem, and the partial least squares method is introduced for brainstem regional signal representation (see Figure 1.5). The proposed framework is applied to investigate functional connectivity of one particular brainstem nucleus – the PPN. Specifically, we investigate the effect of GVS on PPN functional connectivity in PD.

2.1 Introduction

Falls in older adult populations are a significant cause of morbidity and mortality [32] with non-fatal injuries initiating a vicious cycle leading to a fear of falling, social isolation, loss of independence, deconditioning, and a significantly greater use of health care services [165, 193].

In Parkinson’s Disease, gait disturbances such as decreased stride length and gait variability are associated with increased risk of falls. Balance and gait deficits in PD are frequently refractory to therapy [9, 133] and may be actually worsened by pharmacological and surgical
interventions [18], making falls a significant source of morbidity in PD [152]. Freezing of gait (FOG) is a syndrome normally seen in advanced PD and can occur when subjects are either on or off medication. FOG may be partly due to a failure to adequately scale amplitudes for the intended movement [39] and/or defective motor programming setting by the Supplemental Motor Area (SMA) and its maintenance by the basal ganglia, leading to a mismatch between intention and automation [39].

Cognitive and motor function must be carefully integrated to execute gait. Dysfunction of the basal ganglia in PD results in impaired motor control of skilled voluntary movements [104] and movements become excessively slow and underscaled in size [17]. Biochemically, imbalance in multiple neurotransmitters (including but not limited to dopamine, acetylcholine, and GABA) is seen not only in basal ganglia and motor structures, but also limbic circuitries [124, 132]. Balance disturbance and falls in PD may be more related to disruption in cholinergic rather than dopaminergic neurotransmission [20].

A key part of the subcortical cholinergic system is the pedunculopontine nucleus, which appears critically involved in gait disturbances in PD [1, 5, 111], as PPN neuronal loss is evident in PD [138] (Note that although we refer to the PPN throughout this thesis, at the resolution of the imaging used here, it would perhaps be more accurate to refer to this region as the mesencephalic locomotor region as it likely includes the cuneiform nucleushowever, we use PPN as this terminology is consistent with much prior literature (e.g., [1, 5, 111]). Connectivity to/from the PPN appears critical for FOG in PD [61]. Structural deficits in connectivity are evident between basal ganglia-PPN and other tracts in FOG [61, 182]. Diffusion tensor imaging (DTI) tractography obtained with 3T MR imaging in PD patients with FOG has demonstrated asymmetrically decreased connectivity between the PPN and the SMA, compared to PD subjects without FOG [61]. FOG is also associated with diffuse white matter damage involving major cortico-cortical, corticofugal motor, and several striatofrontal tracts with DTI [182]. In addition to structural/anatomical connectivity, advanced neuroimaging techniques have enabled the studies of functional connectivity, which refers to the statistical temporal dependences between anatomically separated brain regions, to reveal the functional communication in the brain. Functional imaging studies (e.g., fMRI) have reported increased activity or altered connectivity during gait visualization in the midbrain locomotion centers between FOG episodes [75], possibly reflecting compensatory mechanisms which might be overwhelmed with stress by turning or multitasking [158]. Moreover, resting-state functional magnetic reso-
nance imaging (rs-fMRI) has allowed the inference of functional connectivity by measuring the level of spontaneous co-activation between fMRI time courses of brain regions recorded during rest. In vivo functional connectivity studies with rs-fMRI suggest that FOG patients may have significantly altered connectivity between PPN-SMA [61], which might reflect a maladaptive compensatory mechanism.

While the PPN has most often been investigated in PD in the context of FOG, it is unclear if altered PPN activity is present in non-FOG PD patients. Surgical targeting of the PPN is usually reserved for people with FOG resulting in significant impairment. Yet, even in early stages of the disease, there are a number of ways in which Parkinsonian gait is different from controls. While mildly affected PD patients can usually perform simple straight-line walk tasks without difficulty, they experience difficulties with turning, and when performing simultaneous motor or cognitive tasks (dual tasks), and/or crossing obstacles [22, 33]. They may have an abnormal gait pattern characterized by a shortened stride length, increased stride variability, and reduced walking speed [27, 116].

Ways to modulate PPN activity and connectivity have proven elusive. Acetylcholinesterase inhibitors may affect the PPN but such effects are likely to be modest. PPN DBS has been shown to (inconsistently) improve gait difficulties in PD [1, 74, 111, 130, 177, 191]. However, the PPN tends to be spatially diffuse and is difficult to visualize on standard T1-weighted images, making electrode placement for DBS therapy difficult. Another potential way to modulate PPN is through the vestibular system, as PPN neurons tend to be highly vestibular-responsive [6]. GVS is a non-invasive technique that activates vestibular afferents to the thalamus and also the basal ganglia [166] which in turn are directed to the PPN [184], possibly explaining why GVS may positively impact posture/standing balance in PD [85]. A few studies have demonstrated that noisy GVS improved postural and balance responses [127, 149] as well as motor deficits in PD [93, 94, 128, 200]. These studies have speculated that noisy vestibular input may have improved information flow through the basal ganglia via stochastic facilitation (SF). SF is a phenomenon observed in a non-linear system where stochastic biological noise paradoxically increases sensitivity of a system to detect a weak stimulus possibly resulting in functional benefits [113]. In addition to noisy stimuli, sinusoidal stimuli have been suggested as a means to activate steady-state, as opposed to transient balance responses that would be induced with pulsed stimuli [91]. Sinusoidally oscillating stimuli may also activate irregular vestibular afferents [69] relying on voltage dependent K-channels [55]. While a couple of fMRI
studies have shown sinusoidal GVS modulated activations in various brain regions [54], GVS’s influence on the PPN has not yet been investigated. A recent study in healthy older adults (n = 20) found that noisy GVS resulted in sustained reduction in Centre of Pressure (COP) parameters, such as velocity, and Root Mean Square (RMS) [68]. The mechanisms of this reduction was speculated to be on the basis of induced synaptic plasticity in the vestibular nuclei and the flocculus of the cerebellum, but effects on the PPN were not considered [68].

Given the non-invasive, and potentially portable nature of GVS, we wished to determine if PPN functional connectivity could be modulated in mildly-affected PD subjects who may demonstrate reduced stride length for example, and thus may be at increased risk for falls, but did not exhibit FOG. Thus, in this chapter, we proposed a novel framework for brainstem–cortical (e.g., PPN–cortical) functional connectivity estimation. We then applied the proposed framework to investigate whether or not functional connectivity between the PPN and other cortical/subcortical regions could be reliably assessed, whether or not these connections were significantly modulated by GVS, and if the connectivity was modulated in a stimulus-specific manner. Since a priori knowledge about functional connectivity to/from the PPN at the spatial and temporal resolution afforded by fMRI is unknown, this was essentially an exploratory approach. Careful care was taken to detect robust activation from PPN structures by analyzing the data in native space (without registration to a template) and utilizing subject-specific weightings of voxels within the PPN region. We demonstrated that PPN functional connectivity is sensitive to vestibular stimulation in PD in a stimulus-dependent manner.

2.2 Methods

2.2.1 Subjects

Twenty-three PD patients (see Table 2.1 for the clinical information) and 12 age-matched healthy controls [5 females; age: 63.3 ± 10.4 (mean ± standard deviation)] participated in the study. The PD patients had mild to moderate PD (Hoehn and Yahr stage IIII) (see Table 2.1) and were scanned at the on-medication state. All participants were recruited from the Pacific Parkinson’s Research Centre (PPRC) at the University of British Columbia (UBC) and provided written, informed consent prior to participation. All studies were approved by the UBC Ethics Review Board. The data were collected in two experiments: in the first group GVS was assessed both ON and OFF L-dopa medication and has been partially reported in a
separate short report [93], and the second group were only assessed in the ON medication state. Only the ON medication studies from the first group are reported here. In the first group, UPDRS scores were assessed in the OFF medication state, while in the second group UPDRS scores were assessed in the ON medication state. A regression model was used to control for these differences (described below).

Table 2.1: Clinical information on PD patients.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Statistics</th>
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<tbody>
<tr>
<td><strong>Characteristics</strong></td>
<td><strong>Statistics</strong></td>
</tr>
<tr>
<td><strong>(mean ± standard deviation)</strong></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>66.4 ± 7.0</td>
</tr>
<tr>
<td>Sex</td>
<td>17 males, 6 females</td>
</tr>
<tr>
<td>UPDRS motor score</td>
<td>22.3 ± 12.4</td>
</tr>
<tr>
<td>UPDRS assessed during on/off</td>
<td>13 on-medication, 10 off-medication</td>
</tr>
<tr>
<td>Hoehn and Yahr stage</td>
<td>1.9 ± 1.0</td>
</tr>
<tr>
<td>LEDD</td>
<td>988.8 ± 798.9</td>
</tr>
</tbody>
</table>

UPDRS = Unified Parkinson’s Disease Rating Scale; LEDD = L-dopa Equivalent Daily Dose.

2.2.2 GVS

Digital signals of the GVS stimuli were first generated on a PC with MATLAB (MathWorks, MA, USA) and were converted to analog signals via a NI USB-6221 BNC digital acquisition module (National Instruments, TX, USA). The analog command voltage signals were then subsequently passed to a bipolar, constant current stimulator (DS5 model, Digitimer Ltd., U.K.). The DS5 constant current stimulator was isolated in the console room with the output cable leading into the scanning room through a waveguide. Along the twisted coaxial output cable, four inductance capacity filters spaced 20 cm apart and tuned for the Larmor frequency (128 MHz) were custom-built. Near the subject, high-resistance radiotranslucent carbon-fiber leads (Biopac Inc., Montreal, Canada) were connected to pre-gelled Ag/AgCl electrodes that were MR-compatible (Biopac Inc., Montreal, Canada). For bilateral stimulation, an electrode was placed over the mastoid process behind each ear. Since the GVS stimuli are alternating current (AC), the anode and cathode are not fixed on one side (as for DC) but they are alternating. The order of GVS condition was kept consistent to be rest, noisy GVS, and sinusoidal GVS across all the participants. The potential caveat of keeping the sequence the
same is the case where there are any post-stimulation effects. To avoid such confounding effects, we allowed a 2-min break between the two GVS conditions. To the best of our knowledge, after-effects of GVS on cortical activation have not yet been investigated. However, we think that the break time was sufficient to avoid after effects based on literature on after-effects of transcranial alternating current stimulation [168].

Since individuals have an inherently subjective perception of GVS, prior to scanning, we determined the individual sensory threshold level (cutaneous sensation at the electrode site) utilizing systematic procedures used in prior GVS studies [81, 178, 192]. We delivered two different types of stimuli at 90% of the individual threshold level: noisy and sinusoidal. The noisy stimulus was zero-mean with 1/f-type power spectrum between 0.1 and 10 Hz and the sinusoidal stimulus was a 1 Hz sine wave.

2.2.3 MRI

Resting-state data were collected on a 3 Tesla scanner (Philips Achieva 3.0T R3.2; Philips Medical Systems, Netherlands) equipped with a 8–channel head coil. During the scanning, all the subjects were instructed to be awake with eyes closed. High-resolution T1 weighted anatomical images were acquired using the following parameters: a repetition time of 1970 ms, echo time of 3.9 ms, inversion time of 1100 ms and flip angle of 15°. BOLD contrast echo-planar (EPI) T2*-weighted images were taken with the following specifications with a repetition time of 1,985 ms, echo time of 37 ms, flip angle of 90°, field of view of 240.00 mm, matrix size of 128 × 128, and with pixel size of 1.9 × 1.9 mm. The duration of each functional run was 8 min for rest condition and 5 min for GVS condition with noisy and sinusoidal stimulus, respectively. As stated above, the order of functional runs was rest, noisy GVS and sinusoidal GVS, and it was kept consistent across all subjects. We allowed 2 min gaps after the noisy stimulus to account for possible post-stimulation effects.

An ROI presumed to include the PPN and cuneiform nucleus was drawn manually on the T1 sequence at the level of the superior cerebellar decussation between medial lemniscus and superior cerebellar peduncle [7, 210] (Figure 2.1). T1 and the fMRI data were registered via FLIRT (with Boundary-Based Registration option) in FSL [161]. The PPN voxels drawn on the T1 were then registered to the fMRI data by applying the inverse transformation calculated by registering the fMRI to the T1 weighted image. After registration, we included neighboring voxels around PPN voxels in our analysis to account for possible partial volume effects.
Figure 2.1: The placement of the PPN ROI on the T1 sequence. The red area represents where the PPN ROI is placed.

2.2.4 Rs-fMRI Preprocessing

The acquired fMRI data were preprocessed using both AFNI and SPM8 software packages. On the whole brain, several preprocessing steps from the AFNI software package were performed. These included despiking, slice timing correction, and 3D isotropic correction (3 mm in each dimension). While the subjects were asked to keep the head still during the scanning session, some head movements occurred during the acquisition process. Motion correction using rigid body alignment was performed to correct for any major head motion during the scan. Besides the fMRI scans, we also collected a T1-weighted structural scan of each of the participants. FreeSurfer was performed on the T1-weighted scans to get the different ROI masks in the T1 space. Each of the subjects’ structural scans was then registered to the fMRI scan using rigid registration. This registration step provided us with the FreeSurfer segmented ROI mask in the fMRI space. All analysis was done in the individual fMRI space rather than transforming all fMRI data to a common template. This was done to prevent introducing any unwanted distortions in the fMRI data by registering it to a common template. In the next step, sev-
eral sources of variance such as head-motion parameters, their temporal derivatives and their squares, white-matter signal, cerebrospinal fluid (CSF) signal were removed using nuisance regression. The fMRI signal was then detrended, and any linear or quadratic trends in the fMRI signal were removed. The signal was then iteratively smoothed until it reached 6 FWHM of smoothness. Finally, bandpass filtering was performed to retain the signal between the recommended frequencies of interest (0.01-0.08 Hz).

Since the brainstem can move independently from the rest of the brain, motion correction on the whole brain motion estimates may not be ideal. Therefore, a separate motion correction of the brainstem was performed. First, the brainstem mask was generated using FreeSurfer on the T1-weighted image of the same subject. The mask was then transferred over to the fMRI using registration as mentioned before. The registered mask was then dilated using a spherical structuring element of radius 3 to incorporate for any errors in the segmentation and registration process. The motion within the brainstem was then corrected independently using the SPM toolbox.

2.2.5 Proposed Framework

The proposed framework for brainstem-cortical functional connectivity estimation is graphically shown in Figure 2.2. Given the fMRI data, we first perform the preprocessing wherein a separate brainstem motion correction is conducted in consideration of the anatomical peculiarities of the brainstem. Next, we design a two-step brainstem-cortical functional connectivity estimation method: the first step is to represent the brainstem regional signal and select a candidate set of cortical and subcortical regions by utilizing Partial Least Squares (PLS), and the second step is to perform a functional connectivity analysis between the brainstem structure and the PLS-selected brain ROIs by utilizing partial correlation. The final output of the proposed framework is then the functional connectivity between the brainstem nucleus and other cortical/subcortical brain regions. Here the studied brainstem nucleus is specifically PPN.

2.2.6 Brainstem Regional Signal Representation and Brain Region Selection

We included 58 ROIs automatically segmented by FreeSurfer as shown in Table 2.2. Two PPN ROIs were manually drawn on the T1-weighted images to include the PPN on each side, namely left PPN and right PPN, respectively. When assessing the functional connectivity between PPN
and other cortical/subcortical brain regions, we first utilized PLS to initially select candidate cortical/subcortical brain regions that significantly covaried with PPN voxels.

PLS is a statistical method that explores the predictive models between predictor variables and response variables \[194\]. It constructs a linear regression model by projecting the predictor variables and response variables to a new set of latent variables the covariance of which is maximized. PLS is particularly useful when the predictor variables are highly collinear, or when the number of predictor variables is larger than that of observations, while classical multiple linear regression models will fail in these cases. PLS has been widely used in various fields of chemometrics, social science, bioinformatics, and neuroscience \[44, 208\].

When applying PLS, we used the 58-ROI dataset as predictor variables, X, and the PPN voxels as response variables, Y, and then tried to predict PPN activity from those 58-ROI time courses.
Table 2.2: The 58 ROIs (in addition to the 2 PPN ROIs) used in the analysis

<table>
<thead>
<tr>
<th>No.</th>
<th>Name</th>
<th>No.</th>
<th>Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Left-Cerebellum-Cortex</td>
<td>30</td>
<td>Right-Cerebellum-Cortex</td>
</tr>
<tr>
<td>2</td>
<td>Left-Thalamus- Proper</td>
<td>31</td>
<td>Right-Thalamus- Proper</td>
</tr>
<tr>
<td>3</td>
<td>Left-Caudate</td>
<td>32</td>
<td>Right-Caudate</td>
</tr>
<tr>
<td>4</td>
<td>Left-Putamen</td>
<td>33</td>
<td>Right-Putamen</td>
</tr>
<tr>
<td>5</td>
<td>Left-Pallidum</td>
<td>34</td>
<td>Right-Pallidum</td>
</tr>
<tr>
<td>6</td>
<td>Left-Hippocampus</td>
<td>35</td>
<td>Right-Hippocampus</td>
</tr>
<tr>
<td>7</td>
<td>Left-Amygdala</td>
<td>36</td>
<td>Right-Amygdala</td>
</tr>
<tr>
<td>8</td>
<td>Left-Accumbens-area</td>
<td>37</td>
<td>Right-Accumbens-area</td>
</tr>
<tr>
<td>9</td>
<td>ctx-lh-caudalanteriorcingulate</td>
<td>38</td>
<td>ctx-rh-caudalanteriorcingulate</td>
</tr>
<tr>
<td>10</td>
<td>ctx-lh-caudalmiddlefrontal</td>
<td>39</td>
<td>ctx-rh-caudalmiddlefrontal</td>
</tr>
<tr>
<td>11</td>
<td>ctx-lh-cuneus</td>
<td>40</td>
<td>ctx-rh-cuneus</td>
</tr>
<tr>
<td>12</td>
<td>ctx-lh-entorhinal</td>
<td>41</td>
<td>ctx-rh-entorhinal</td>
</tr>
<tr>
<td>13</td>
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<td>42</td>
<td>ctx-rh-inferiorparietal</td>
</tr>
<tr>
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<td>ctx-lh-inferiortemporal</td>
<td>43</td>
<td>ctx-rh-inferiortemporal</td>
</tr>
<tr>
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<td>44</td>
<td>ctx-rh-lateralorbitofrontal</td>
</tr>
<tr>
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<td>ctx-rh-medialorbitofrontal</td>
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<td>46</td>
<td>ctx-rh-middletemporal</td>
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<td>47</td>
<td>ctx-rh-parahippocampal</td>
</tr>
<tr>
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<td>ctx-lh-paracentral</td>
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<td>ctx-rh-paracentral</td>
</tr>
<tr>
<td>20</td>
<td>ctx-lh-postcentral</td>
<td>49</td>
<td>ctx-rh-postcentral</td>
</tr>
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<td>ctx-lh-posteriorcingulate</td>
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<td>ctx-rh-posteriorcingulate</td>
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<td>51</td>
<td>ctx-rh-precentral</td>
</tr>
<tr>
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<td>ctx-lh-precuneus</td>
<td>52</td>
<td>ctx-rh-precuneus</td>
</tr>
<tr>
<td>24</td>
<td>ctx-lh-rostralanteriorcingulate</td>
<td>53</td>
<td>ctx-rh-rostralanteriorcingulate</td>
</tr>
<tr>
<td>25</td>
<td>ctx-lh-rostralmiddlefrontal</td>
<td>54</td>
<td>ctx-rh-rostralmiddlefrontal</td>
</tr>
<tr>
<td>26</td>
<td>ctx-lh-superiorfrontal</td>
<td>55</td>
<td>ctx-rh-superiorfrontal</td>
</tr>
<tr>
<td>27</td>
<td>ctx-lh-superiorparietal</td>
<td>56</td>
<td>ctx-rh-superiorparietal</td>
</tr>
<tr>
<td>28</td>
<td>ctx-lh-superiortemporal</td>
<td>57</td>
<td>ctx-rh-superiortemporal</td>
</tr>
<tr>
<td>29</td>
<td>ctx-lh-insula</td>
<td>58</td>
<td>ctx-rh-insula</td>
</tr>
</tbody>
</table>

The general model for PLS is

\[
X = TP^T + E \tag{2.1}
\]

\[
Y = UQ^T + F
\]

where X is a t-by-m matrix of ROI data (predictor variables), with t corresponding to the
number of time points, and $m (= 58)$ representing the number of subject-independent (non-PPN) ROIs; $Y$ is a $t$-by-$n$ matrix of PPN voxel time courses (response variables), where $n$ is the number of PPN voxels (which was subject-dependent); $T$ and $U$ are, respectively, $t$-by-$c$ component matrices decomposed from $X$ and $Y$ ($T$ and $U$ are also called X Score and Y Score, respectively), where $c$ is the number of components; $P$ is an $m$-by-$c$ loading matrix of ROI dataset, and $Q$ is an $n$-by-$c$ loading matrix of PPN voxels; and $E$, $F$ are the $t$-by-$m$ and $t$-by-$n$ matrices, respectively, representing error terms. Essentially, PLS performs the decompositions of $X$ and $Y$ to maximize the covariance between $T$ and $U$.

We then interrogated the loadings of the $X$ components (i.e., the columns of $P$) to determine if they were significantly different from zero across subjects. The same procedure was conducted for both left PPN and right PPN, respectively, and then the union set of the selected regions from left PPN and right PPN was used as the final candidate set of brain regions. In addition, we used the first component of $Y$ (i.e., the first column of $Y$ Score) to represent the PPN regional signal in the subsequent functional connectivity analyses.

### 2.2.7 Functional Connectivity Analyses

We further performed functional connectivity analyses between the PPN and PLS-derived regions. Functional connectivity measures were obtained by computing the partial correlation coefficients between the represented PPN signal, which was obtained by the PLS analysis, and the averaged time courses of each PLS-derived region. We conducted the functional connectivity analyses on a subject-by-subject and task-by-task basis. Specifically, for each subject, functional connectivity was assessed for each of the three conditions, i.e., rest, noisy and sinusoidal GVS conditions, by taking the time courses for each condition time segment of interest from the PPN and PLS-derived regions and computing the partial correlation coefficients between them. For simplicity, we summed the absolute values of the significant connectivity coefficients from both left and right PPN to get an overall PPN functional connectivity.

To investigate whether or not functional connectivity between the PPN nuclei and PLS-derived regions was significantly affected by GVS, we calculated overall PPN functional connectivity differences between GVS on (i.e., noisy/sinusoidal GVS condition) and GVS off (i.e., rest condition). An independent one-sample t-test was then performed on the calculated connection coefficient differences across subjects, with the null hypothesis that the difference was zero, to determine if significant connectivity changes were induced by GVS.
2.3 Results

2.3.1 Brain Region Selection

The PLS analysis results found 10 ROIs in the PD group and 5 ROIs in the control group that significantly covaried with PPN voxels ($p < 0.05$). In the PD group, the ROIs included the cerebellum cortex, hippocampus, amygdala, inferior parietal, middle temporal, and precuneus regions on the left, and the pallidum, hippocampus, amygdala, and middle temporal on the right (Figure 2.3). In the control group, the caudate on the left, and the caudate, entorhinal cortex, inferior temporal, and parahippocampal regions on the right were associated with PPN activity (Figure 2.4).

Figure 2.3: PLS-derived ROIs in the PD group that are significantly covaried with PPN voxels. The detected 10 regions, including cerebellum cortex, hippocampus, amygdala, inferior parietal, middle temporal, and precuneus regions on the left, and the pallidum, hippocampus, amygdala, and middle temporal on the right, are marked with different colors.
2.3.2 Functional Connectivity Analyses

To determine the effect of the LEDD on connectivity and to correct for the fact that some subjects had their UPDRS assessed off medication, we performed a regression analysis where connection strengths across subjects was the dependent variable, and LEDD, UPDRS score, whether or not the UPDRS was done on or off medication were independent variables. We then evaluated the regression coefficients for the LEDD to determine if it had a significant effect on overall PPN functional connectivity, which it did not ($p > 0.05$).

The functional analysis results demonstrated that GVS differently affected overall PPN functional connectivity in PD and control groups. In the control group, no significant differences in overall PPN functional connectivity were found between GVS on (i.e., noisy/sinusoidal GVS condition) and GVS off (i.e., rest condition). In the PD group, the overall magnitude of PPN functional connectivity correlated negatively with UPDRS scores ($r = -0.39$, $p = 0.035$,
Both noisy and sinusoidal GVS increased the magnitude of overall PPN functional connectivity \( (p = 6 \times 10^{-5} \text{ and } 3 \times 10^{-4}, \text{ respectively, Figure 2.6}) \). Furthermore, in order to determine if overall connectivity of the PPN was particularly related to postural instability, we also compared the connectivity to the retropulsion test score from the UPDRS (Figure 2.5, inset). Since our emphasis was on early patients, we could not perform a statistical analysis, as there was only 1 subject with a score of 2 and 1 subject with a score of 4. However, as shown in Figure 2.5, and consistent with overall UPDRS scores, there was a trend toward decreased connectivity with higher retropulsion test scores.

Figure 2.5: The correlation relationship between the overall PPN functional connectivity and UPDRS scores in the PD group. The inset shows the relationship between the overall PPN functional connectivity and the retropulsion test scores from the UPDRS.

Although both types of stimuli augmented overall PPN functional connectivity (both positive and negative connectivity as shown in Figure 2.6), in order to determine if there are differences between the types of stimuli, we performed a post-hoc analysis to determine which PPN connections were most influential in determining changes in overall connectivity. Specifically, we performed t-tests on each connection to determine if the different types of GVS stimuli
increased or decreased connectivity between the PPN and other brain regions.

![Graph showing overall PPN connectivity differences between GVS conditions.](image)

**Figure 2.6:** The overall PPN functional connectivity differences between GVS on (i.e., noisy and sinusoidal GVS condition) and GVS off (i.e., rest condition) in the PD group.

For the left PPN, noisy GVS decreased connectivity with the right pallidum and sinusoidal GVS increased connectivity with the left inferior parietal region (Table 2.3 and Figure 2.7). For the right PPN, noisy GVS decreased connectivity with the left cerebellar cortex, increased connectivity with the right amygdala and increased connectivity with the left inferior parietal region; sinusoidal GVS decreased connectivity with the left amygdala (Table 2.4 and Figure 2.8). Note that only the connection between left PPN and left inferior parietal region would survive multiple comparisons.

<table>
<thead>
<tr>
<th>Connectivity</th>
<th>Type of Stimuli</th>
<th>t-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Right pallidum</td>
<td>Noisy GVS</td>
<td>-2.32</td>
<td>0.015</td>
</tr>
<tr>
<td>Left inferior parietal</td>
<td>Sinusoidal GVS</td>
<td>2.81</td>
<td>0.005</td>
</tr>
</tbody>
</table>

Table 2.3: Statistics on significant left PPN functional connectivity changes induced by GVS stimuli in the PD group
2.4 Discussion

Balance impairment remains a vexing problem in PD and is associated with considerable morbidity. Pharmacological (particularly dopaminergic) and surgical interventions have had varying degrees of success. Development of novel therapies has also been challenging because the
Figure 2.8: The GVS impact on the connectivity between the right PPN and PLS-derived regions in the PD group. The red areas (with three different color levels indicating the different levels of significance values) represent the regions significantly affected by noisy GVS. The green areas represent the regions significantly affected by sinusoidal GVS. The blue areas represent the regions with no significant changes. The corresponding ROI names and significance values are labeled in the figure.

pathophysiology and neuropathological substrates underlying gait disturbances are incompletely known.

To the best of our knowledge, we have shown for the first time that is possible with GVS to non-invasively modulate the functional connectivity in PD subjects between cortical/subcortical ROIs and the PPN – a structure critical for normal supraspinal control of locomotion. This demonstrated alteration in functional connectivity complements previous work examining anatomic connectivity patterns. Anatomically, the PPN has been shown to have connections with various areas such as the vestibular nuclei [6], deep cerebellar nuclei [78], premotor, SMA and primary motor cortices [7], frontal eye fields [109], thalamic nuclei and basal ganglia nuclei [38]. Widespread projections involving the PPN include direct glutamatergic inputs from the motor cortex, and GABAergic inputs from substantia nigra pars
reticulata (SNr), globus pallidus internus (GPi), subthalamic nucleus (STN), and deep nuclei of cerebellum. Ascending efferent projections target GPi, substantia nigra pars compacta (SNc), and thalamus. Descending efferent projections connect to pontine, medullary reticular formation, and the spinal cord vital for control of muscle tone and locomotion. Additionally, the PPN appears to be important in the initiation, acceleration, deceleration, and termination of locomotion through connections to the basal ganglia and higher cortical regions [92].

Our results indicate significant differences in the PPN functional connectivity between PD subjects and controls. This is particularly relevant when noting that none of our PD subjects had FOG, given their relatively mild disease. However, even mild disease is associated with altered gait and our results suggest that PPN functional connectivity patterns change even in the early stages of disease course.

We have shown that both GVS stimuli patterns (noisy and sinusoidal) augment overall deficient PPN functional connectivity in PD. Our results are consistent with previous studies demonstrating GVS activation of vestibular afferents to basal ganglia [166, 184], which are also directed to the midbrain locomotion network [130]. PET studies in humans have also shown activation in the putamen in response to vestibular stimulation [24].

We found differences in PPN network connectivity depending upon the type of stimulus used. Noisy GVS significantly decreased the functional connectivity between the left PPN and right-pallidum in PD. The PPN receives strong inhibitory, GABAergic inputs from the BG nuclei (GPi, STN, and SNr), which have disrupted connectivity in PD [126]. In particular, previous studies suggest that the PPN may be the principal target of pallidal outflow, since more than 80% GPi neurons were found to send axonal branches to both the PPN and thalamus in monkeys [76]. In PD, the inhibitory GABAergic synaptic activities from the GPi to the PPN is abnormally overactive, which may underlie the akinesia and the gait problems seen in the PD [126]. Taken together, these studies demonstrate the important role of the PPN and pallidal connectivity in motor and gait dysfunctions of PD. We demonstrated that noisy GVS significantly decreased connectivity between the left PPN and right-pallidum in PD (but not normal controls), which suggests that potential benefits of GVS on balance in PD may be partly mediated through attenuation of overactive pallidal inputs to the PPN.

Noisy GVS also decreased connectivity between the right PPN and the left cerebellar cortex. A diffusion-weighted imaging study found the connectivity of the PPN region with the cerebellum, thalamus, pallidum, and STN [120]. The cerebellum functions that help control of
movement, coordination, and posture \[133\] are speculated to be associated with the existence of the pathway with the PPN \[120\]. The fact that prior studies have suggested a beneficial effect of noisy GVS \[68\] may indicate that hyperactive PPN-cerebellar connections are partly normalized with GVS.

We also found that GVS increased the functional connectivity between the left inferior parietal cortex and the right PPN (with noisy stimuli) and left PPN (with sinusoidal stimuli). Previous studies in non-human primates examining cortical inputs to the pontine nuclei have supported the anatomical and functional relationships between the PPN and the inferior parietal cortex \[108, 110\]. Like the PPN, the left inferior parietal cortex is involved in gait as well as visuospacial information processing, motor planning, and preparation \[35\]. Imagining normal gait activates the left inferior parietal lobule, in addition to the precuneus and bilateral dorsal premotor cortex, the left dorsolateral prefrontal cortex, and the right posterior cingulate cortex \[105\]. The left inferior parietal cortex appears to be especially related to FOG. Gray matter volume in the left inferior parietal region is significantly reduced in PD subjects with FOG patients compared to both PD subjects without FOG and healthy controls \[90\]. Thus, our result that noisy GVS increased the connectivity between the PPN and the left inferior parietal cortex might suggest GVS could improve gait difficulties in PD by augmenting the connectivity.

In the current study, one of the advantages is that we performed analyses keeping each subject’s data in their original space without warping the data to a common template. We are frankly skeptical of fMRI studies suggesting robust activation from brainstem structures (e.g., PPN) when data are spatially transformed to a template, given the significant registration errors that can occur to small brainstem nuclei during whole-brain registration \[121\]. In addition, we utilized PLS to find the combination of PPN voxels on a subject-by-subject basis that maximally corresponded with other ROIs. In effect, the first column of Q in Equation 2.1 represents a subject-specific spatial filter to focus the activity that maximally covaried with other ROIs.

It is interesting that many of the abnormal connectivities that we detected are lateralized, when balance might be considered a midline function. Balance control and gait are asymmetrical in patients with PD, and gait asymmetries have been linked to the pathophysiology of FOG \[23\]. The symptoms of PD generally show an asymmetric onset and progression and it has been proposed that this may lead to a degree of unbalanced motor function, such that FOG is triggered by a breakdown in the bilateral co-ordination underlying the normal timing of gait \[135\].
There are a few limitations in our study. We examined a relatively small number of PD patients. However, by carefully selecting the PPN voxels via PLS on a subject-by-subject basis, we expect that we have significantly enhanced our effect size, thus increasing our statistical power. PLS is one of the most widely used blind source separation (BSS) approaches which have largely benefited the neuroscience studies [44, 45, 162, 208]. In the future studies, we are interested to further explore the effective voxel selections using such data-driven approaches. We have shown GVS induced changes in PPN functional connectivity in people with mild to moderate PD but not in healthy controls. We speculate an inverted-U shape of effectiveness of GVS as a function of disease severity: in controls, GVS had minimal effect, in early/moderate PD, it had some effect (shown here), and in severe disease, degeneration in the PPN itself may prevent modulation of its connectivity. Future work is required to further investigate the relationship between disease severity and PPN functional connectivity and determine the behavioral significance of this altered PPN functional connectivity. We do note that we found a negative correlation between UPDRS scores and overall PPN functional connectivity, yet still found robust modulation of connectivity across all of our subjects.

The relation between behavioral gait measures (e.g., gait variability) and ultimate falls risk - the most important issue for people with PD - is an active area of research. Conceivably, previously-described GVS improvements in balance may not ultimately translate into reduced fall risk but such determination would require a prospective trial in the future. We have focused on the PPN because of its possible therapeutic implications, but gait disturbances in PD likely involve several cortical and subcortical structures. For example, PD patients have decreased activity of the SMA during gait [75], and PD individuals have diminished pre-movement electroencephalographic potentials originating from the SMA prior to step initiation [160]. Future studies to assess connectivity changes modified by GVS in other supra-spinal locomotion centers including SMA/pre-SMA may help to guide the development of optimal stimuli on a subject-specific basis.

2.5 Conclusion

In this chapter, we proposed a novel framework for brainstem-cortical functional connectivity estimation. The proposed framework was applied to investigate the effect of GVS on PPN functional connectivity in PD. Our results suggested that GVS can enhance deficient PPN
functional connectivity seen in PD in a stimulus-dependent manner. This may provide a mechanism through which GVS assists balance in PD, and may provide a biomarker to develop individualized stimulus parameters.
Chapter 3

PLS-based Regional Signal Representation for PPN Functional Connectivity Estimation in PD: fMRI Effect of Walking Exercise

In Chapter 2, we propose a novel framework to assess brainstem-cortical functional connectivity. By extension, in this chapter, we further apply the proposed framework to perform treatment evaluation of another therapeutic intervention. Specifically, we investigate the effect of walking exercise on PPN functional connectivity in PD (see Figure 1.5).

3.1 Introduction

As mentioned in Chapter 2, gait disturbances in Parkinson’s disease such as decreased stride length and gait variability, and especially FOG, are associated with increased fall risk and hence a significant source of disability [152]. Falls have devastating impacts on the quality of life of individuals with PD, and often trigger a downward spiral of frailty and can lead to depression, social isolation, activity avoidance, and fear of falling [18]. Unfortunately, gait impairment, including FOG, falls and postural instability (PI) are currently largely untreatable in PD [133]. New therapeutic approaches, as well as finding potentially new targets for intervention are desperately needed.

The neuropathological substrates underlying postural and gait impairment in PD are poorly understood. Pre-motor, primary motor and supplementary motor cortical areas, and the cerebellum and basal ganglia all modulate brainstem structures, such as the mesencephalic locomotor center (MLC), that generate postural responses and influence equilibrium, balance and
A key component of the MLC is the PPN, a brainstem cholinergic structure with widespread connectivity to other brain regions. PPN receives direct glutamatergic inputs from the motor cortex, and GABAergic inputs from substantia nigra, GPi, STN, and deep nuclei of the cerebellum. Ascending efferent projections from the PPN target Gpi, substantia nigra pars compacta, and thalamus, and descending efferent projections target pontine and medullary reticular formation and spinal cord vital for control of muscle tone and locomotion. Altered connectivity to/from the PPN is likely a factor in PD gait abnormalities such as FOG, as impaired structural connectivity can be demonstrated between basal ganglia-PPN and other tracts in FOG. As suggested in previous studies, significantly stronger functional connectivity between PPN-SMA may have been found in FOG patients, reflecting possibly maladaptive compensatory mechanisms. As the PPN is affected in PD, there has been intense interest on how to modulate PPN activity, but results have been inconsistent. The PPN is modestly affected by acetylcholinesterase inhibitors. Multiple studies on PPN-DBS have suggested possible clinical improvement in PD patients, although results have been varied. Reasons for this response variability may be difficulties in determining precise DBS electrode placement, and variability of functional and structural connectivities from and to the PPN. In Chapter 2, we have demonstrated that PPN functional connectivity can be modified by GVS in PD subjects, but not in controls, with changes in functional connectivity correlating with UPDRS score.

Behaviorally, exercise, physiotherapy as well as other measures targeting stepping-in-place have been shown effective interventions for PD gait impairment and in preventing falls, but the effects of these interventions on PPN functional connectivity are unknown. Ambulosono is one such program, that uses an iPod for music via Bluetooth headphones, and in some cases, assessing stride length, and if this drops below an individually designated level, the music stops playing. The music is designed to improve step automaticity, increase the physical vigour of lower limb movements, and enhance voluntary and automatic motor control.

In this chapter, we utilized resting-state fMRI to determine if functional connectivity between the PPN and other cortical/subcortical brain regions could be modulated by Ambulosono in PD patients with mild gait impairment. Since there is increasing recognition that a number of cortical and subcortical regions may be important for gait difficulties in PD, we specifically employed a hypothesis-driven approach examining PPN functional connectivities, because it is potential target in DBS and to prevent problems with massive multiple comparisons that
would be required for a fully exploratory approach. Similar to Chapter 2, careful care was taken to ensure robust activation from PPN structures by analyzing the data in native space (without registration to a template) and utilizing subject-specific weightings of voxels within the PPN region. We hypothesized that PPN functional connectivity could be modified by a music walking training approach, in a dose-dependent manner.

3.2 Materials and Methods

3.2.1 Subjects

Twenty-seven PD subjects (Table 3.1) with mild to moderate PD (HY I - III) and optimally treated with PD medications were recruited from the Pacific Parkinson’s Research Centre at the University of British Columbia (UBC). The study was approved by the UBC Ethics Review Board, and all subjects gave written, informed consent prior to participation.

<table>
<thead>
<tr>
<th>Clinical characteristics</th>
<th>Statistics (mean ± standard deviation)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>64.5 ± 8.1</td>
</tr>
<tr>
<td>Gender</td>
<td>17 males, 10 females</td>
</tr>
<tr>
<td>Disease duration (years)</td>
<td>4.9 ± 4.0</td>
</tr>
<tr>
<td>UPDRS III</td>
<td>23.9 ± 11.3</td>
</tr>
<tr>
<td>H &amp; Y</td>
<td>1.4 ± 0.5</td>
</tr>
<tr>
<td>FES-I</td>
<td>20.1 ± 5.7</td>
</tr>
<tr>
<td>FOG-Q</td>
<td>2.8 ± 4.0</td>
</tr>
<tr>
<td>MoCA</td>
<td>27.0 ± 3.1</td>
</tr>
<tr>
<td>Total number of walks</td>
<td>29.8 ± 17.2</td>
</tr>
<tr>
<td>Total length of training (min)</td>
<td>1471.7 ± 849.4</td>
</tr>
<tr>
<td>Average walking (m/min)</td>
<td>73.1 ± 18.5</td>
</tr>
<tr>
<td>Total walking distance (km)</td>
<td>112.4 ± 71.7</td>
</tr>
</tbody>
</table>

Values are given as mean ± standard deviation. UPDRS III = Unified Parkinson’s Disease Rating Scale-Part III; HY = Hoehn and Yahr stage; FES-I = Falls Efficacy Scale International; FOG-Q = Freezing of Gait Questionnaire; MoCA = Montreal Cognitive Assessment.
3.2.2 Ambulosono

The Ambulosono device and protocol have been previously described at length [47]. In brief, music with high emotional salience and likeableness are chosen and used to induce a high level of physiological arousal and as means to activate locomotor networks and heighten sensorimotor perception, awareness and self-efficacy during walking [42,148]. Later, music play may be made contingent upon the amplitudes of walking steps so that smaller walking steps, i.e., shuffling, can be prevented before it becomes an undesirable habit [47]. However, for the purposes of this study, we pooled the groups who listened to music with and without the contingency to increase our sample size.

The study timeline and protocol are shown in Figure 3.1. The walking program first started with a baseline walking of 4 weeks, two of which they walked with NO music followed by two weeks of music listening while walking. Participants were instructed to walk a minimum of 20 minutes/walk, 3 times per week or a total of 60 minutes/week, at their own preferred pace. During the training, participants were required to upload their walking files once a week via wireless internet connection to our online database. The statistics on the walking data is shown in Table 3.1.

All 27 patients completed clinical assessments, including UPDRS-III, HY, the MoCA for general cognitive functioning, and self-reported questionnaires including the FES-I survey for fear of falling, the FOG-Q for freezing at baseline, and then after Ambulosono training for comparison of pre- and post-intervention. MRI was performed at baseline and 3 months post-music training. Patients were in medication ON state during the clinical assessment and MRI scan. Five patients did not have a repeat UPDRS-III at follow-up point. Therefore, they were excluded from the UPDRS-related analysis.

3.2.3 fMRI Data Acquisition and Preprocessing

The parameters of data acquisition and fMRI preprocessing steps were the same as those in Section 2.2.3 and 2.2.4. The scan duration of each functional run was 8 mins at the resting-state.
3.2.4 Brainstem Regional Signal Representation and Brain Region Selection

Here we basically used the same brainstem-cortical functional connectivity modelling framework as in Chapter 2 (Figure 2.2). Initially, 80 ROIs were included in this study as shown in Table 3.2. Additionally, two PPN ROIs (namely left PPN and right PPN respectively) as mentioned above were incorporated to conduct the PPN functional connectivity analysis. Prior to the functional connectivity estimation, PLS was utilized to select a subset of cortical/subcortical ROIs that significantly covaried with PPN voxels [30]. The dominant component of PPN voxel dataset was utilized to represent the PPN regional signal in the subsequent functional connectivity analysis.

3.2.5 Functional Connectivity Analyses

Functional connectivity network structure was additionally assessed between the PPN and PLS-selected ROIs using the $PC_{fdr}$ algorithm [97], a conditional independence based network...
Table 3.2: The PLS-selected ROIs (italic) from the 80 ROIs used in this study

<table>
<thead>
<tr>
<th>No.</th>
<th>Name</th>
<th>No.</th>
<th>Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
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<td>Right-Cerebellum-Cortex</td>
</tr>
<tr>
<td>2</td>
<td>Left-Thalamus- Proper</td>
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<td>Right-Thalamus- Proper</td>
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<tr>
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<td>Left-Caudate</td>
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<td>Right-Caudate</td>
</tr>
<tr>
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<td>Right-Putamen</td>
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<td>Right-Pallidum</td>
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<td>Right-Amygdala</td>
</tr>
<tr>
<td>8</td>
<td>Left-Accumbens-area</td>
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<td>ctx-rh-parahippocampal</td>
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<td>48</td>
<td>ctx-rh-insula</td>
</tr>
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<td>49</td>
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<td>ctx-rh-postcentral</td>
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<td>74</td>
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<td>L-PMd</td>
<td>78</td>
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</tr>
<tr>
<td>40</td>
<td>L-PMv</td>
<td>80</td>
<td>R-PMv</td>
</tr>
</tbody>
</table>

structure learning approach [72], and suitably modified to incorporate a false discovery rate (FDR) control procedure, which was set to be 0.05.

We utilized a common structure approach [41], which imposes the same connectivity network structure on each subject, while allows the connectivity strength (coefficients) to be different
across subjects and tasks. The strength/coefficients of functional connectivity were estimated by computing partial correlation coefficients on a subject-by-subject basis.

3.2.6 Statistical Analyses

The individual connection coefficients of PPN functional connectivity and the sum of absolute values of significant connectivity coefficients were compared between pre-exercise and post-exercise using a paired t-test. Associations between PPN functional connectivity and UPDRS score and walking data were investigated using Pearson’s correlation.

3.3 Results

Using PLS, a total of 74 ROIs were significantly covaried with the PPN (p < 0.01, FDR corrected - Table 3.2). There was significant functional connectivity between the left caudate and left posterior cingulate and the left PPN (p < 0.05, FDR corrected). For the right PPN, significant functional connectivity with right hippocampus and left inferior parietal were found (p < 0.05, FDR corrected), as shown in Figure 3.2.

3.3.1 PPN Functional Connectivity Changes after Ambulosono Exercise

Ambulosono significantly increased the magnitude of overall right PPN functional connectivity (Figure 3.3a, p = 0.02, FDR corrected), but showed a trend in decreasing the overall PPN functional connectivity at the left side (Figure 3.3a, p > 0.05). The connectivity between right PPN and left inferior parietal was significantly increased (p = 0.03, FDR corrected, Figure 3.3b), and the right PPN functional connectivity with right hippocampus was moderately increased (p = 0.07, FDR corrected, Figure 3.3b).

3.3.2 PPN Functional Connectivity Correlates with Clinical Scores

There was a significant decrease in UPDRS-III score after Ambulosono exercise (p = 0.003, Figure 3.3c). The overall functional connectivity of left PPN positively correlated with the baseline UPDRS score (Figure 3.3d, r = 0.36, p = 0.05). In addition, the magnitude of the decreased left PPN functional connectivity by exercise strongly correlated with the improvement in UPDRS-III score (Figure 3.3d, r = 0.63, p = 0.001). In contrast, the right PPN functional connectivity was increased after Ambulosono. There was no clear correlation between the
increase in right PPN functional connectivity and the improvement in UPDRS, a trend, however, was seen that individuals with lower baseline UPDRS showed higher magnitude of increase in right PPN functional connectivity (not significant). There was a decrease in the left PPN functional connectivity that correlated with the total number of walks (Figure 3.3e, \( r = 0.43, p = 0.02 \)) and the total length of training (Figure 3.3f, \( r = 0.39, p = 0.03 \)).

3.4 Discussion

We have shown that walking exercise affects PPN functional connectivity in PD, in a dose-dependent manner. Previously, it has been shown that PPN functional connectivity can be modified under certain conditions. For instance, PPN connectivity can be activated by treadmill training [2]. Unilateral PPN-DBS during self-paced lower limb movements results in increases in regional cerebral blood flow in interconnected structures of cerebello-thalamo-cortical circuit [11] including the PPN region. Chronic low frequency PPN-DBS can modify brain connectivity in FOG, resulting in reduction of corticopontine overactivity seen in the pre-stimulation period [155]. In our study we found multiple cortical, subcortical and brainstem structures that significantly covaried with the PPN (Table 3.2), consistent with previous studies examining functional and anatomic connectivity patterns [7, 30, 38, 183]. The demonstrated alteration of PPN functional connectivity by Ambulosono complements our observation in Chapter 2 of GVS also modulating PPN functional connectivity in PD patients [30].

We have demonstrated that Ambulosono improved PD motor symptoms as measured by UPDRS-III. This is compatible with prior observations that rhythmic auditory clues [8] and mental singing during walking may improve gait in PD [151], and numerous studies demonstrating overall benefits of physical activity in PD [50].

In our study, left and right PPN connectivities were different at baseline, and were differently affected by Ambulosono exercise. After exercise, we found significant increases in overall right PPN functional connectivity, more or less independent of disease severity. In contrast, before the intervention, the functional connectivity of the left PPN positively correlated with the baseline UPDRS-III score, so that even though overall left PPN functional connectivity was reduced in the PD population, greater connectivity was seen in greater disease severity. There are two potential explanations for this apparent paradox. One is that the left PPN functional connectivity represents a compensatory response that increases with disease severity. Note that
with a compensatory mechanism, reduction of disease effects (as evidenced by the improvement in behavioral measures) will also result in a decrease in compensatory mechanisms. Another relates to neural efficiency, whereby exercise results in less co-activation of ancillary brain regions, presumably due to more efficient recruitment of necessary resources to complete the task of ambulation [122]. In contrast, with the right PPN, while connectivity with both the left inferior parietal and right hippocampus were increased after exercise, this did not correlate with changes in motor function. While it is still unclear whether there is a dominant PPN in humans, especially in PD gait-impaired individuals, our result further supports an asymmetry in PPN functional connectivity, as has been suggested in Chapter 2.

There have been several studies that have demonstrated altered connectivity between left and right PPN. In PD patients with FOG, reduced white matter connectivity can be seen from the PPN to the cerebellar locomotor regions, thalamus, and multiple regions of the frontal and prefrontal cortex only in the right hemisphere of freezers [62]. Functional connectivity in postural instability gait difficulty (PIGD) subgroups have increased functional connectivity between the left PPN and the SMA-proper, while the right PPN is hyper-connected to the right premotor cortex and left M1. Tremor dominant (TD) subgroups have increased functional connectivity between the left PPN and the left premotor cortex, pre-SMA and SMA [183]. Greater functional connectivity between the SMA and MLC can be positively correlated with freezing severity in FOG [61]. The pattern of such enhanced connectivity does not appear to behave like a useful compensatory role, but rather may contribute to FOG. It is difficult to compare these results directly with ours, as the group of subjects described in this report did not have FOG, given their relatively mild disease. However, even mild PD is associated with altered gait, and our results suggest that the PPN functional connectivity patterns we observed can be seen even in the early stages of disease.

There are a few limitations in our study. Similar to Chapter 2, we studied a relatively small number of PD patients. Still, by carefully selecting the PPN voxels via PLS on a subject-by-subject basis, we expect that we have significantly enhanced our effect size, thus increasing our statistical power. Furthermore, we would include patients with more advanced disease when gait and balance concerns are more prominent (i.e. with FOG). In severe disease, degeneration in the PPN itself may prevent modulation of its connectivity. In addition, combining with structural connectivity studies, for instance with tractography, we may be able to establish imaging biomarkers to predict responders from non-responders to treatment interventions. We
did not have a control group; we believe there is ample evidence already showing the beneficial effects of exercise, particularly walking, so we therefore examined the dose-response of such an intervention.

### 3.5 Conclusion

In this chapter, we extended the application of the brainstem-cortical functional connectivity estimation framework proposed in Chapter 2, to investigate the effect of walking exercise on PPN functional connectivity in PD. We demonstrated that PPN functional connectivity can be modulated by walking exercise in a dose-dependent manner. The results provided additional evidence of altered functional connectivity of the locomotor network in PD patients, and further supported evidence of asymmetric PPN functional connectivity early in the disease course. The observed modification in PPN functional connectivity provided a potential mechanism to explain how exercise may improve gait function, possibly by increasing neural efficiency.
Figure 3.2: The functional connectivity of the left and right PPN. The connected regions of
the left PPN include left caudate and left posterior cingulate, and the connected regions of the
right PPN include right hippocampus and left inferior parietal.
Figure 3.3: The PPN functional connectivity changes due to the Ambulosono exercise and related relationships with the UPDRS score. (a) The Ambulosono exercise significantly increased the magnitude of overall right PPN functional connectivity, but showed a trend to decrease left PPN functional connectivity. (b) For individual PPN functional connectivity, the Ambulosono exercise significantly increased the right PPN functional connectivity with left inferior parietal, and also moderately increased the connectivity between the right PPN and right hippocampus. (c) The Ambulosono exercise significantly decreased the UPDRS score, suggesting an improvement in PD patients overall motor symptoms by the music walking exercise. (d) The baseline left PPN functional connectivity correlated positively with UPDRS score, suggesting that the lower connectivity indicates a better overall motor function. Additionally, the decrease in the left PPN functional connectivity strongly correlated with the improvement in UPDRS score after Ambulosono training. (e) The decrease in the left PPN functional connectivity correlated with the number of walks. (f) The decrease in the left PPN functional connectivity correlated with the training time. (#p > 0.1, *p < 0.1, **p < 0.05, ***p < 0.005)
Chapter 4

Constrained Canonical Correlation Analysis for Brain Functional Connectivity Estimation

In ROI-based brain functional connectivity, representing the signal in a given ROI is an important issue. With most current approaches, the signals from same-ROI voxels are simply averaged, neglecting any inhomogeneity in each ROI and thus less optimally reflecting ongoing activity in the ROI. In this chapter, we develop a novel method of representing regional signal with local brain activity incorporated and estimating brain functional connectivity via constrained CCA method (see Figure 1.5). We then apply the proposed method to estimate cortical/subcortical functional connectivity using Human Connectome Project data.

4.1 Introduction

Inferring brain functional connectivity from fMRI data has advanced our understanding of large-scale functional coordination of the brain. As mentioned in Section 1.2, a key issue for assessing the ROI-based brain functional connectivity is how to represent the information in a given ROI. Although receiving relatively little attention (in large part due to the ease of simply taking the average signal from same-ROI voxels), regional signal representation is a general research problem worthy of careful investigation. This is because different ROI signal representation strategies may significantly impact the subsequent results inferred from fMRI data, as has been previously demonstrated [176]. Current methods include using average signal, PCA and selecting a “representative” voxel. However, these methods ignore the possible functional inhomogeneity of ROIs and may exhibit poor reliability [115, 150, 181]. Recently, it has been suggested that connectivity analyses can be prone to inaccuracy when there is insufficient
spatial constraint to ensure that the voxels with concentrated activity within each ROI are appropriately emphasized [48]. Therefore, improved brain functional connectivity modelling methods that incorporate both the regionally-specific nature of brain activation (in the context of relation to a specific ROI) and the appropriate assessment of representative voxels are required to guide the fMRI analysis.

We propose a new method for the estimation of brain functional connectivity from fMRI data, in which appropriate handling of within-ROI clusters of voxels are taken into account for constructing the ROI representative signal, and the connections between ROIs are appropriately inferred. This method is implemented in two steps, where regional activity is first detected by density clustering and then spatially-constrained canonical correlation analysis (CCA) is applied to the identified regionally-specific activity structures. Density clustering [139] is a recently proposed algorithm that divides data into clusters based only on the distance (which can be defined in temporal and/or spatial domains) between data points. This method has advantages of fast speed and automatic detection of the number of clusters by finding density peaks. We utilize this density clustering algorithm to explore local brain activation patterns within an ROI, dividing the voxels into several groups which maximize intra-group homogeneity. We then utilize a modified version of CCA, a well-known multivariate statistical method for maximizing the correlation relationship between two sets of variables and previously used in fMRI [65]. Here we propose a novel approach, which we call local activity constrained CCA (LA-cCCA), by modifying the traditional CCA method from two aspects: first by including spatial dominance constraints on highly locally-connected voxels to ensure the results more accurately reflect the spatial data structure, and second, by imposing non-negativity constraints that only allows for non-negative combinations of voxels to ease interpretability. We utilize LA-cCCA to simultaneously construct ROI representative signals and estimate the connections between ROIs.

The remainder of this chapter is organized as follows: Section 4.2 introduces density clustering and spatially constrained CCA algorithms, followed by the integration of the two algorithms. Section 4.3 demonstrates the results using both synthetic and real fMRI data, and our results are compared with two main ROI signal representation approaches, i.e., the average signal approach and the PCA approach. Finally, Section 4.4 provides the discussion of the present study.
Figure 4.1: A schematic overview of the proposed method. Take two ROIs as an illustration, each ROI is first divided into several functional sub-regions (painted by different colors) by density clustering, and meanwhile the most important voxel for each ROI is identified. Next, the detected most important voxels and their neighbours (pink) are selected to delineate the ROIs. Finally, spatially constrained CCA is performed between the ROIs wherein the constructed ROI representation signals (red) exhibit maximized correlation: the weights of the blue ones (non-selected voxels) are zero, and the weights show an increase from red to yellow. The most important voxels have maximum weights (yellow).
4.2 Methods

In this section, we introduce the proposed LA-cCCA method — a schematic overview of the method is shown in Figure 4.1. Given two ROIs, we first detect the regional brain activation patterns by density clustering, and then apply spatially constrained CCA on the basis of the regionally-specific nature of brain activity to estimate the connection strength between the pair of ROIs. This is then repeated for all other pairs. In the next subsections, the method will be described in detail. We start with the introduction of density clustering algorithm, followed by the formulation of spatially constrained CCA for brain functional connectivity estimation, and finally give a comprehensive description on the integration of the two algorithms.

4.2.1 Density Clustering

Rodriguez and Laio [139] introduced a novel clustering algorithm for fast location of density peaks, which we refer to here as density clustering. This algorithm considers cluster centers as exhibiting two properties: (1) higher density than their neighbouring data points and (2) large distance from other data points with higher densities. For each data point, two core quantities are calculated on the basis of distance matrix between data points. One is the density value $\rho_i$ of data point $i$, which is computed using a Gaussian kernel as

$$\rho_i = b \sum_{j=1}^{N} \exp\left(-\frac{(d_{ij} - c)^2}{d_c^2}\right)$$  (4.1)

where $d_{ij}$ denotes the distance between two data points $i$ and $j$, and $d_c$ is a pre-specified cutoff distance. As suggested in [139], the parameters $b$ and $c$ are set to 1 and 0 respectively, and $d_c$ is chosen such that the average percentage of neighbours is 1%. It can be seen that the data point with a high density value tends to have short distances to other data points. In this study, the distance is defined based on functional dissimilarity between voxels (described below). Therefore, the voxel that is functionally similar to other voxels tends to have a high density value.

The other measure, $\delta_i$, is calculated as the minimum distance between the data point $i$ and any other data point with higher density

$$\delta_i = \min_{j, \rho_i < \rho_j} (d_{ij})$$  (4.2)
For the data point with the highest density, the corresponding $\delta_i$ is taken as $\delta_i = \max_j (d_{ij})$. It is noteworthy that $\delta_i$ tends to be larger for those data points corresponding to local or global maxima of density.

Therefore, the cluster centers are identified as the data points with both high density $\rho$ and high distance $\delta$. In this study, the distance matrix is defined based on functional connectivity (e.g., Pearson’s correlation as described below) between voxels, the cluster centers thus correspond to the voxels that are local connectivity hubs which maintain the strongest connections over a neighbourhood. Then cluster assignment is performed by assigning each of the rest data points to the cluster that its nearest neighbour of higher density belongs to. The advantages of this algorithm include fast speed, relatively simple implementation where only the distance matrix is required, a one-step cluster assignment (as opposed to an iterative method) and automatic detection of the number of clusters.

**Geodesic Distance**

The distance matrix used in the proposed method is based on *geodesic distance* [80]. In order to obtain the geodesic distance, we first generated a weighted graph where each voxel within an ROI corresponded to the vertices, and the edges were constructed between spatially neighbouring voxels (within a $3 \times 3 \times 3$ cube) by computing the Pearson’s correlation distance. The geodesic distance is computed as the shortest path length through the weighted graph between each pair of voxels. Therefore, both the functional dissimilarity and spatial distance between voxels are reflected in the geodesic distance.

**Automatic Density Peak Detection**

In density clustering [139], cluster centers are manually selected from a decision graph - a scatter plot of the density value $\rho$ and the distance $\delta$ (illustrated below). The data points corresponding to density peaks, i.e., the data points with both high $\rho$ and large $\delta$, are chosen as cluster centers. However, it is both time consuming and laborious to conduct manual selection, especially for batch processing. Therefore, it is desirable to incorporate an automatic cluster center selection into the original algorithm. In this chapter, we performed automatic density peak detection via optimizing the silhouette index [187]. The criterion used for the automatic selection of cluster centers conforms with the aforementioned fact that the cluster centers correspond to the data points with high $\rho$ and large $\delta$, which are typically located in the upper right corner of the
decision graph. The specific procedure for the automatic density peak detection is described in Algorithm 1.

**Algorithm 1 Automatic density peak detection**

1. Define a set $C$ as possible values for the number of clusters $c_n$, and a value $\alpha$ such that $\rho_\alpha$ denotes the $\alpha$-th quantile of the density values.
2. for $n = 1, 2, \cdots, N$ do
3. Among the data points $\mathcal{I} = \{i : \rho_i > \rho_\alpha\}$, select the data points with the top $c_n$ highest distance values $\delta$ as cluster centers;
4. Perform cluster assignment as in Rodriguez and Laio’s algorithm [139];
5. Calculate the average silhouette index $s_n$;
6. end for
7. The clustering configuration with the maximum average silhouette index is the final cluster model, including the number of clusters, the corresponding cluster centers and the cluster assignment.

### 4.2.2 Spatially Constrained CCA

CCA is applied on two data sets and tries to maximize the correlation between them by linear transformations. Specifically, given two sets of variables $X = [x_1, x_2, \cdots, x_p]$ and $Y = [y_1, y_2, \cdots, y_q]$, with the matrix dimension of $t \times p$ and $t \times q$ respectively where $t$ is the number of observations and $p, q$ represent the number of variables, CCA seeks the weight vectors $w_x$ and $w_y$

\[
x = w_{x,1}x_1 + w_{x,2}x_2 + \cdots + w_{x,p}x_p = Xw_x
\]  

\[
y = w_{y,1}y_1 + w_{y,2}y_2 + \cdots + w_{y,q}y_q = Yw_y
\]

so that $x$ and $y$ have the maximum correlation, as shown in Equation (4.5).

\[
\max_{w_x, w_y} C_{x,y} = \frac{w_x^t X^t Y w_y}{\sqrt{w_x^t X^t X w_x} \sqrt{w_y^t Y^t Y w_y}}
\]  

(4.5)

This problem can be solved by the following eigenvalue problems:

\[
(X^t X)^{-1}(X^t Y)(Y^t Y)^{-1}(Y^t X)w_x = C_{x,y}^2 w_x
\]  

(4.6)
\[(Y'Y)^{-1}(Y'X)(X'X)^{-1}(X'Y)w_y = C^2_{x,y}w_y. \] (4.7)

Therefore, the weight vectors \(w_x\) and \(w_y\) correspond to the eigenvectors associated with the largest eigenvalues of \((X'X)^{-1}(X'Y)(Y'X)\) and \((Y'Y)^{-1}(Y'X)(X'X)^{-1}(X'Y)\) respectively.

CCA has proved useful for examining neuronal activity in fMRI [48, 65]. In this chapter, we utilize the CCA method to find representative signals from brain ROIs. Based on the definitions above, suppose \(X\) and \(Y\) denote fMRI signals from two brain ROIs, then the linear combinations \(Xw_x\) and \(Yw_y\) obtained from the CCA method can be used as the ROI representation signals, in the way that the connections between them are detected in a sensitive way. Note that a given ROI will have several weight vectors, corresponding to the other ROIs. This is consistent with the notion that the very important voxels within a given ROI may be a function of what other ROI is being considered in a pairwise interaction.

Conventional CCA can result in both positive and negative weights, but the negative weights can be difficult to interpret. We therefore imposed non-negativity constraints on the weight vectors so that the resulting representative signals \(Xw_x\) and \(Yw_y\) can be considered as a weighted mean of the original time series within the ROI. Besides the obvious non-negativity constraint, it is also critical to incorporate spatial constraints on the weight vectors to take into account the connectome properties of voxels which ensures adequate spatial weighting of the most locally-connected voxels (i.e., the local connectivity hubs that maintain the strongest connections over a neighbourhood) to reduce sensitivity to artifact.

The constraints that we imposed on the weight vectors in the CCA can be summarized as,

\[w_i \geq \max_{j \neq i} w_j \geq 0,\] (4.8)

where \(w_i\) represents the weights for the most locally-connected voxels within a ROI, and \(w_j\) denotes the weights for other voxels in the same ROI. The spatial constraint for one ROI, say \(X\), can be written as \(D_xw_x \geq 0\). For instance, suppose the voxel \(x_1\) be the most locally-connected
voxel, then the $p \times p$ matrix $D_x$ can be defined as

$$D_x = \begin{bmatrix}
1 & 0 & 0 & \cdots & 0 \\
1 & -1 & 0 & \cdots & 0 \\
1 & 0 & -1 & \cdots & 0 \\
\vdots & \vdots & \vdots & \ddots & \vdots \\
1 & 0 & 0 & \cdots & -1
\end{bmatrix}$$  \hspace{1cm} (4.9)

We further formulated this spatially constrained CCA as a constrained optimization problem

$$\max_{w_x, w_y} C_{x,y} = w_x'X'Yw_y$$ \hspace{1cm} (4.10a)

subject to

$$D_x w_x \geq 0 \hspace{1cm} (4.10b)$$

$$D_y w_y \geq 0 \hspace{1cm} (4.10c)$$

$$w_x \geq 0 \hspace{1cm} (4.10d)$$

$$w_y \geq 0 \hspace{1cm} (4.10e)$$

$$w_x'X'Xw_x = 1 \hspace{1cm} (4.10f)$$

$$w_y'Y'Yw_y = 1 \hspace{1cm} (4.10g)$$

Note that we adapted the objective function in the CCA in the way that the denominator in the definition of the correlation between $x$ and $y$ as in Equation 4.5 has been converted into the normalization terms that have been incorporated in the equality constraints as Equations 4.10f and 4.10g.

We utilized interior point method to solve the spatially constrained CCA problem. Interior point method is a certain class of algorithms used for solving constrained optimization problems, which has proven powerful for nonlinear programming [123]. By implementing the interior point method, the original constrained optimization problem is converted to a sequence of approximate problems through a barrier function. Given the constrained minimization problem
The approximate problem is written as

\[
\begin{align*}
\min_{x, s} & \quad f(x) - \mu \sum_i \log s_i \\
\text{s.t.} & \quad h(x) = 0 \\
& \quad g(x) + s = 0
\end{align*}
\] (4.12a)

where \( \mu \) is a positive scalar, which is called barrier parameter, and \( s_i \) is slack variables, the number of which is equal to that of inequality constraints. The logarithm term in Equation 4.12a is called a barrier function. The interior point method consists of solving the approximate problem for a sequence of positive barrier parameters \( \{\mu_k\} \). As \( \mu \) decreases to zero, the solution of the approximate problem should approach the solution of the original constrained optimization problem.

The approximate problem of spatially constrained CCA in Equation 4.10 is

\[
\begin{align*}
\min_{w, s} & \quad f(w) - \mu \sum_i \log s_i = \min_{w, s} -w^T Z w - \mu \sum_i \log s_i \\
\text{s.t.} & \quad g_1(w) + s_1 = -D w + s_1 = 0 \\
& \quad g_2(w) + s_2 = -w + s_2 = 0 \\
& \quad h_1(w) = w^T A w - 1 = 0 \\
& \quad h_2(w) = w^T B w - 1 = 0
\end{align*}
\] (4.13a)

where \( w = [w^T_x, w^T_y]^T \), \( s = [s_1^T, s_2^T]^T \). The matrices \( Z, D, A \) and \( B \) can be defined accordingly to be consistent with Equation 4.10.
The Lagrange function associated with the approximate problem is then

\[ L(w, s, \lambda, \gamma) = -w' Z w - \mu \sum_i \log s_i + \sum_i \lambda_i (g_i(w) + s_i) + \sum_j \gamma_j h_j(w) \] (4.14)

where \( g(w) = \begin{bmatrix} g_1(w) \\
g_2(w) \end{bmatrix} \), \( h(w) = \begin{bmatrix} h_1(w) \\
h_2(w) \end{bmatrix} \), and \( \lambda, \gamma \) are Lagrange multiplier vectors associated with \( g(w) \) and \( h(w) \) respectively.

The Karush-Kuhn-Tucker (KKT) conditions (i.e., the first-order necessary conditions for a constrained optimization problem) are

\[
\begin{align*}
\nabla f(w) + J_g^T \lambda + J_h^T \gamma &= 0 \\
S\lambda - \mu e &= 0 \\
g(w) &\leq 0 \\
h(w) &= 0 \\
\lambda &\geq 0
\end{align*}
\] (4.15a,b,c,d,e)

where \( J_g, J_h \) denote the Jacobian matrices of the constraint functions \( g \) and \( h \), respectively, \( S \) is defined to be the diagonal matrix with diagonal entries given by the vector \( s \), and \( e = (1, 1, \ldots, 1)^T \).

Applying Newton’s method to Equation 4.15 we obtain the variable update \((\Delta w, \Delta s, \Delta \lambda, \Delta \gamma)\)

\[
\begin{bmatrix}
H & 0 & J_g^T & J_h^T \\
0 & S\Lambda & 0 & -S \\
J_h & 0 & I & 0 \\
J_g & -S & 0 & I
\end{bmatrix}
\begin{bmatrix}
\Delta w \\
\Delta s \\
-\Delta \gamma \\
-\Delta \lambda
\end{bmatrix}
=
\begin{bmatrix}
\nabla f(w) - J_g^T \lambda - J_h^T \gamma \\
S\lambda - \mu e \\
h \\
g + s
\end{bmatrix}
\] (4.16)

where \( H \) denotes the Hessian of the Lagrange function \( L \), i.e., \( H = \nabla^2 f(w) + \sum_i \lambda_i \nabla^2 g_i(w) + \sum_j \gamma_j \nabla^2 h_j(w) \), and \( \Lambda \) is the diagonal matrix with the diagonal entries given by the vector \( \lambda \).

We used backtracking line search to determine the step size \( \alpha \), which decreases the merit
function

\[ \phi(w, s; \nu) = f(w) - \mu \sum_i \log s_i + \nu \| g(w) + s \|_1 \]

\[ + \nu \| h(w) \|_1 \]

(4.17)

where the penalty parameter \( \nu \) may increase with the iteration number to ensure the solution towards feasibility [123]. The convergence criterion for each approximate problem (inner loop) and the overall optimization (outer loop) is measured using the following error function, which is based on the fulfillment of the KKT conditions.

\[ E(w, s, \lambda, \gamma; \mu) = \max \{ \| \nabla f(w) + J_g^T \lambda + J_h^T \gamma \|_\infty, \]

\[ \| S\lambda - \mu e \|_\infty, \| h(w) \|_\infty, \| g(w) + s \|_\infty \} \]

(4.18)

The interior point algorithm for spatially constrained CCA is described in Algorithm 2.

**Algorithm 2** The iterative algorithm of the line search interior point method

1: Initialize \( w_0, s_0, \lambda_0, \gamma_0 \) and select the parameter \( \sigma \in (0, 1) \).
2: while Convergence is false do
3: \hspace{1em} while Convergence is false do
4: \hspace{2em} Calculate the variable update \( (\Delta w, \Delta s, \Delta \lambda, \Delta \gamma) \) using Equation (4.16);
5: \hspace{2em} Determine the step size \( \alpha \) using backtracking line search and the merit function in Equation (4.17);
6: \hspace{2em} Update \( (w^+, s^+, \lambda^+, \gamma^+) \leftarrow (w + \alpha \Delta w, s + \alpha \Delta s, \lambda + \alpha \Delta \lambda, \gamma + \alpha \Delta \gamma) \);
7: \hspace{1em} end while
8: \hspace{1em} Set \( \mu \leftarrow \sigma \mu \);
9: end while

4.2.3 Integration of Regional Activity Structure into Network Modelling

The proposed LA-cCCA method essentially integrates regional brain activity, via density clustering, into spatially constrained CCA for bivariate interaction estimation. Specifically, given a set of ROIs, our method can be summarized as follows (see Figure 4.1):

1. Due to local functional segregation, one ROI can encompass several sub-ROIs. Therefore, we first utilized density clustering to detect regional activity on brain ROIs, dividing each ROI into several functionally homogeneous and spatially contiguous sub-ROIs and identifying the most important voxel (i.e., the voxel corresponding to the density peak) for each sub-ROI.
2. To recognize the fact that the true fMRI activation tends to occur in spatially connected clusters rather than a single voxel, and to avoid the arbitrary of the use of a single voxel and increase robustness, we include the neighbouring voxels around each density peak into the analysis. In other words, we select a set of voxels (i.e., the heavily locally-connected voxels and their neighbours) to delineate each ROI.

3. Based on the selected voxels, we perform spatially constrained CCA between ROIs, in which the spatial dominance constraint is imposed on the most important voxels and the non-negativity constraint is introduced on all voxels. In this step, the ROI representative signals are constructed and the connections between the ROIs are estimated.

4.3 Results

4.3.1 Synthetic Data Set

We validated our method on the synthetic data set by comparing the performance of the proposed method with that of two other popular correlation based brain functional connectivity network estimation methods, which represent the ROI signal by either the PCA or the average signal.

Two different scenarios were considered to test the performance of the proposed method. In the first scenario, we considered each ROI as having just one underlying signal, i.e., the ROI was functionally homogeneous across all voxels. Three ROIs were generated for the connectivity assessment. In the second scenario, we considered the fact that one ROI can encompass several functional sub-regions. In this case, we generated two ROIs, one with two functional sub-regions, and the other with just one underlying signal. In both scenarios, the underlying signals were first generated from Gaussian distributions. Then signals for all voxels within the ROI were generated according to the signal to noise ratio (SNR), which is defined as

\[
SNR = \frac{\sigma^2_{signal}}{\sigma^2_{noise}}
\]

(4.19)

where \(\sigma^2\) is the variance of the data.

The correlation coefficients between the underlying signals were generated from a uniform distribution on the interval [0,1]. The connectivity was identified as between those ROIs with significant correlations (\(p\)-value < 0.05). We then applied the proposed LA-cCCA, as well
as PCA and average-signal based correlation method to estimate the connectivity between the generated ROIs. In each method, the significance of the connectivity was determined by performing 5000 permutations, and setting the significance level to 0.05. The performance of each method was evaluated by the square root of mean square error (RMSE) as a function of SNR. The RMSE is defined as

$$RMSE = \sqrt{\frac{1}{n} \sum_{i=1}^{n} (v_i - \hat{v}_i)^2}$$

(4.20)

where $v$ is the “true” connectivity vector with $n$ elements, and $\hat{v}$ is the estimated connectivity vector with the same length. To obtain a reliable assessment, the procedure was repeated 50 times for each method. The average performance over 50 runs was compared between different methods.

The results for the two scenarios on the synthetic data are shown in Figure 4.2 and 4.3. For each figure, the upper panel shows examples of the true connectivity matrix and the estimated results by the three methods, and the lower panel compares the performances of the different methods. As can be seen from the figures, in both cases, the proposed LA-cCCA model performed consistently better among the three methods across different SNRs. The average signal method exhibited a moderate performance, and the PCA method performed least well. As expected, the performance of each method was mostly improved with increased SNRs. In the second scenario, the advantage of LA-cCCA method was more apparent, suggesting the proposed method is more powerful in the case of the existence of sub-ROIs.

4.3.2 Real fMRI Data Set

Subjects and Data Acquisition

A total of 100 subjects from the Wu-Minn Human Connectome Project (HCP) [179] were employed in this study. All subjects were healthy subjects aged 22-35 years. The MRI data from all subjects were collected on a customized Siemens 3T “Connectome Skyra” scanner equipped with a 32–channel head coil and a “body” transmission coil using HCP’s acquisition protocols [180]. Structural images were obtained with a three-dimensional MPRAGE T1-weighted sequence with repetition time of 2400 ms, echo time of 2.14 ms, inversion time of 1000 ms and flip angle of 8°. Two resting-state fMRI sessions were acquired on separate days while subjects were fixed on a cross hair and asked to keep eyes open. The fMRI data scanning parameters
Figure 4.2: Simulation results for the first scenario. (a) The true connectivity matrix, as well as the estimated connectivity matrices by average signal, PCA and LA-cCCA methods, where the blue entries correspond to the connectivities that are not considered in the analysis. (b) The performances of the three methods in terms of RMSE, as a function of SNR.
Figure 4.3: Simulation results for the second scenario. (a) The true connectivity matrix, as well as the estimated connectivity matrices by average signal, PCA and LA-cCCA methods, where the blue entries correspond to the connectivities that are not considered in the analysis. (b) The performances of the three methods in terms of RMSE, as a function of SNR.
were as follows: repetition time of 720 ms, echo time of 33.1 ms, flip angle of 52°, field of view of 208 mm × 180 mm, matrix size of 104 × 90, 72 slices, 2 mm isotropic voxels and 1200 time points.

Data Preprocessing

The data were processed by the HCP minimal preprocessing pipeline [71], including denoising, motion correction and alignment to standard space. FSL, FreeSurfer, and the Connectome Workbench software packages were employed by this pipeline. Specifically, three pipelines, namely PreFreeSurfer, FreeSurfer and PostFreeSurfer were used to process the structural data, and two pipelines, namely fMRIVolume and fMRISurface, were used to process the fMRI data. Furthermore, fMRI time series were de-meaned and linearly detrended, and the whole brain signal was regressed out. Finally, the fMRI data were spatially smoothed by a 2 mm FWHM Gaussian kernel and bandpass filtered at 0.01 Hz to 0.08 Hz.

Table 4.1: The ROIs used in this study

<table>
<thead>
<tr>
<th>No.</th>
<th>Label</th>
<th>Name</th>
<th>No.</th>
<th>Label</th>
<th>Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>L1</td>
<td>Left Accumbens</td>
<td>2</td>
<td>R1</td>
<td>Right Accumbens</td>
</tr>
<tr>
<td>3</td>
<td>L2</td>
<td>Left Amygdala</td>
<td>4</td>
<td>R2</td>
<td>Right Amygdala</td>
</tr>
<tr>
<td>5</td>
<td>L3</td>
<td>Left Caudate</td>
<td>6</td>
<td>R3</td>
<td>Right Caudate</td>
</tr>
<tr>
<td>7</td>
<td>L4</td>
<td>Left Diencephalon Ventral</td>
<td>8</td>
<td>R4</td>
<td>Right Diencephalon Ventral</td>
</tr>
<tr>
<td>9</td>
<td>L5</td>
<td>Left Hippocampus</td>
<td>10</td>
<td>R5</td>
<td>Right Hippocampus</td>
</tr>
<tr>
<td>11</td>
<td>L6</td>
<td>Left Pallidum</td>
<td>12</td>
<td>R6</td>
<td>Right Pallidum</td>
</tr>
<tr>
<td>13</td>
<td>L7</td>
<td>Left Putamen</td>
<td>14</td>
<td>R7</td>
<td>Right Putamen</td>
</tr>
<tr>
<td>15</td>
<td>L8</td>
<td>Left Thalamus</td>
<td>16</td>
<td>R8</td>
<td>Right Thalamus</td>
</tr>
</tbody>
</table>

“L” represents Left and “R” represents Right.

Brain Functional Connectivity Network Estimation

We applied the proposed method to assess the brain functional connectivity network for each subject, utilizing the ROIs shown in Table [4.1]. We first performed density clustering on each ROI, providing a decision graph that plotted $\delta$ as a function of $\rho$ for each voxel within the ROI. The voxels located in the upper right corner of the decision graph were identified as density peaks. Instead of manual selection, the density peaks were automatically detected according to Algorithm 1. Specifically, we set the candidate number of density peaks as $C = \{2, \ldots, 6\}$ and $\alpha = 10\%$ to prevent the voxels with small $\rho$ but high $\delta$ (i.e. outliers) being selected as density peaks.
peaks, as previously suggested [187]. The optimal number of density peaks was determined by the maximum silhouette index. Figure 4.4 shows one example of the decision graph and the process of determining the number of density peaks on the region of putamen. As can be seen from the decision graph, the density peaks should correspond to the two voxels in the upper right corner. Consistently, during the process of automatic density peak selection, the maximum silhouette index was found at the cluster number of 2, suggesting the voxels (with $\rho_i > \rho_{\alpha}$) with the top two largest $\delta$ as density peaks. As shown in Figure 4.5, the putamen was functionally segregated into two sub-regions with associated density peaks.

![Decision Graph and Silhouette Index](image)

Figure 4.4: Example of decision graph (left) and the process of automatically determining the number of clusters (right).

In the next step, we selected density peaks and their neighbours in each ROI for brain functional connectivity network estimation. Spatially constrained CCA was performed between ROIs to assess the connectivity, with the spatial dominance constraint on density peaks and the non-negativity constraint on all selected voxels. It should be mentioned that we initially estimated a fully connected brain network. Due to the relatively high computational cost, we did not perform a permutation test to determine the significance of the connectivity as in
Figure 4.5: Density clustering on the Putamen. (a) The spatial distribution of subcortical volume in which 16 ROIs are included. (b) The region of Putamen. (c) Two functional subregions and their corresponding peak voxels detected by density clustering algorithm.
the simulation study. Instead, we determined the significant connectivity via a thresholding strategy (described below). Example of brain functional connectivity network estimated by the proposed method is shown in Figure 4.6.

Figure 4.6: A fully connected brain network estimated by the proposed LA-cCCA method for one subject. (a)-(c) The sagittal, axial and coronal view of subcortical brain regions, where each node represents one ROI. (d) The estimated fully connected brain network, where the lines represent the connections between ROIs. The strength of the connection increases from blue to red.

Reproducibility

To demonstrate the performance of the proposed method, we compared the LA-cCCA method with the average signal and PCA method in terms of reproducibility of the estimated brain networks between two sessions. We hypothesized that the better the network modelling method is, the more reproducible the estimated brain networks are. For the LA-cCCA method, the brain functional connectivity network was estimated as described above. For the other two methods, we first computed the average signal or the first principal component of each ROI to represent the ROI signal. The connectivity was then estimated by calculating the correlation coefficients.
between ROIs using their ROI representative signals. For each subject, the brain functional connectivity network was assessed across two different sessions, each on separate days using the three methods (Figure 4.7). The connectivity matrices estimated by the LA-cCCA method exhibited more consistent patterns between the two sessions, followed by the average signal method, but the PCA method performed the worst. The reproducibility was then quantified by measuring the Euclidean distance between the two connectivity matrices for each subject. The smaller the distance is, the more reproducible the connectivity pattern is. For the initially estimated fully connected networks, the LA-cCCA method demonstrated the most reproducible results, the average signal method showed a moderate reproducibility, and the PCA method was the least reproducible (Figure 4.8(a)). In real applications, we may need to determine the significance of the estimated connectivity. Thresholding is a commonly used method for detecting significant connectivities. Here the threshold was chosen such that the connection density (i.e., the number of existing connections divided by the maximum possible number of connections) reached a desired value. To fully evaluate the proposed method, we examined the performance using a range of connection densities. The results showed that the proposed LA-cCCA method consistently outperformed the other two methods across different density
Figure 4.8: Comparison of the reproducibility for different methods. (a) The reproducibility on the fully connected brain networks. (b) The reproducibility across different levels of connection density.
levels in terms of the reproducibility (Figure 4.8(b)).

4.4 Discussion and Conclusion

In this chapter, we have proposed a novel approach for brain functional connectivity estimation, which simultaneously determines the optimal ROI representation signals when considered in context of connectivity with another ROI. Application of this method on both simulated and real fMRI data set resulted in higher accuracy of brain network estimation and/or a more reproducible connectivity pattern.

We utilized density clustering to investigate the local activity within the ROI. There are two parameters in the algorithm: a cutoff distance $d_c$ for calculating the density and a threshold $\alpha$ for preventing the outliers ($\rho_i < \rho_\alpha$) being selected as density peaks. The parameters in this chapter were chosen according to heuristics suggested in previous studies [139, 187], although we observed that mild perturbation in the parameter values didn’t appear to have a significant impact on the results. The proposed method has advantage of detecting functional sub-regions in the ROI and incorporates such local activity into the brain functional connectivity estimation. For a fair comparison with the other two methods, we didn’t split each sub-region in the real data application. However, this advantage could facilitate potential analysis of sub-network interactions in the brain.

We consider “reproducibility” as one key metric to evaluate the brain network models in this study. Our results suggest that the proposed method outperformed the average-signal and PCA based correlation methods in terms of the reproducibility. Previous studies on the comparisons of different ROI signal representation methods suggested the PCA method tended to be more sensitive to functional inhomogeneity and exhibited a worse reproducibility than the average signal method [181], consistent with our current work. We utilized the Euclidean distance as the reproducibility measure in this study, which could be expanded for a comprehensive comparison of different metrics in future work.

One of the advantages of the LA-cCCA method is that we adaptively learn the weight vector for a single ROI according to its paired ROI. ROIs, especially if they are defined anatomically, may, and likely will, exhibit functionally inhomogeneous activations, depending upon neural context. Therefore, we specifically allow, with the same spatial constraints, different weight vectors for the same ROI depending upon which other ROIs are being considered. This inherent
flexibility will ameliorate any concerns about functional heterogeneity within an ROI. As an illustration, we analyzed the relationship between the variation in the weight vector of each ROI and the size of the ROI. The variation in the weights was characterized by the average standard deviation over all voxels, and the size of the ROI was measured by the number of voxels in the ROI. Unsurprisingly, the result showed that the variation in the weights was positively correlated with the ROI size (Figure 4.9, correlation coefficient = 0.73, p-value = 0.04), suggesting that the larger the ROI is, the more heterogeneous the regional activation is, and likewise, the smaller the ROI is, the more homogeneous the regional activation tends to be.

![Figure 4.9: Correlation between the variation in the weights and the size of ROI.](image)

In summary, the proposed method provides a potential method for regional activity representation and presents a more reliable model for connectivity network estimation. In the future studies, quantification of brain functional connectivity networks via graph theoretical measures could further facilitate interpretations. Extension of the method to dynamic analyses may also be of interest, and application to the neurological diseases could reveal disease related connectivity patterns, providing potential biomarkers.
Chapter 5

Graph Theory Method for Dynamic Brain Functional Connectivity Analysis

In Chapter 2, 3 and 4, we have addressed the problems of regional signal representation and brain functional connectivity modelling associated with ROI-based brain functional connectivity. After estimating brain functional connectivity networks, a consequent challenging problem is how to summarize the information from the inferred brain functional connectivity networks, particularly in the dynamic setting which contains rich spatiotemporal information. In this chapter, we propose the use of dynamic graph theoretical measures to extract useful information from time-varying brain functional connectivity networks as potential MRI-related biomarkers (e.g., the newly introduced graph spectral measure, Fiedler value, see Figure 1.5). Specifically, we apply the graph theory method to study the dynamics of cortical/subcortical functional connectivity in PD.

5.1 Introduction

As one of the most common degenerative neurological disorders, Parkinson’s disease affects a large population worldwide, particularly in people over 50 years of age. It is characterized by the cardinal motor features of tremor, rigidity, slowness of movement and postural instability, as well as various cognitive and behavioral problems, such as depression, sleep disturbances and dementia. The relatively high prevalence and serious consequences of PD have a significant impact on global health. Traditionally the diagnosis of PD has been clinical, because there can be overlap with other conditions, and traditional structural imaging methods have been relatively uninformative, compared to other brain diseases (e.g. stroke, cancer).
Functional neuroimaging technologies, such as resting-state fMRI, may prove diagnostically and prognostically useful for a number of brain diseases including PD. Due to its non-invasiveness, relatively high spatial resolution, and lack of requirement for the subject to engage in a task, resting-state fMRI has been adopted widely.

If brain regions are considered as ‘nodes’ and the interactions between brain regions as ‘edges’, graph theoretical analysis can be used to characterize the architecture and information flow in brain networks [142]. Altered graph theoretical properties have been reported in neurological diseases, such as AD and schizophrenia [14, 170], but a relative paucity of such studies exist for PD [3, 131].

In conventional functional connectivity analysis, brain networks are typically estimated utilizing the full time series under the assumption that connectivity is temporally stationary. However, even at the temporal resolution of fMRI, it is clear that different brain regions intrinsically interact and coordinate in a dynamic manner [82], and functional connectivity dynamic properties may provide insight into fundamental properties of brain networks [82, 99]. A body of literature has thus been dedicated to investigating dynamic functional connectivity, with a list of proposed network modelling methods that include a sliding window approach [82], a change point detection approach [49], time-frequency analysis [198] and hidden Markov models [136].

Assessing the topological properties of these dynamic functional connectivity networks may offer a promising avenue for evaluating brain dynamics at a system level [63] as evidenced by recent studies in schizophrenia and mild cognitive impairment [189, 202]. We suggest that the application of dynamic graph measures to PD will provide us deeper insights into the underlying mechanisms of PD.

The first aim of this study is to compare such dynamics in healthy controls (HCs) and PD subjects by computing a range of graph measures over the time-varying functional connectivity networks. To construct the time-varying functional connectivity networks that can reveal the dynamic brain functional connectivity patterns, we use the sliding window approach, wherein the sparse inverse covariance matrix is computed from the windowed segments [4].

The second aim is to investigate two promising graph spectral measures that have received little attention in the study of PD: the Fiedler value and the normalized Fiedler value. The Fiedler value, also known as algebraic connectivity, reflects the global connectivity of a graph [59, 134], and has been applied in network science as an indicator of how well connected the
network is \[102\]. The normalized Fiedler value is similar to the Fiedler value, but normalized for the node degree in a graph \[134\]. These two measures are derived from the spectral graph theory, or often referred to as Laplacian eigenmaps. In the latest literature, the information provided by Laplacian eigenmaps, especially by the second smallest eigenvalue of the Laplacian (the Fiedler value), has been successfully applied for the brain parcellation \[100, 106\]. Two other studies have utilized the Fiedler value to characterize structural connectivity networks \[51, 134\]. Here we are the first to explore the potential of Fiedler value for studying the dynamics of functional connectivity in PD.

The third aim is to examine the utility of dynamic graph measures as potential features for contributing to an MRI biomarker of PD. Conventional analysis of fMRI data exploring group differences between healthy individuals and neurological patients have commonly used univariate approaches \[30, 117\]. However, these studies have limited practical usage, as they only show significances at a group level. Thus, there has been an increasing interest in alternative forms of analysis – e.g., applying machine learning techniques to fMRI data for effective and accurate computer-aided disease diagnosis \[87, 141, 209\], that provide discriminative power at the individual level. Previous studies that performed discriminative analysis of PD using machine learning methods have used features such as structural/functional connectivity, ALFF (amplitude of low-frequency fluctuations) or ReHo (regional homogeneity) \[46, 103\]. In this study, we propose utilizing dynamic graph measures derived from a temporal series of functional connectivity networks. To the best of our knowledge, this is the first work to utilize dynamic graph measures, especially the Fiedler value, for PD classification.

The remainder of this chapter is organized as follows: Section \[5.2\] provides an extensive description on the dataset and methods used in this study, including the dynamic functional connectivity estimation, graph theoretical analysis and statistical analysis. The classification framework using dynamic graph measures is also presented in this section. Section \[5.3\] presents the dynamic graph theoretical analysis results, demonstrates the classification performance of the proposed framework, and provides discussion. Finally, section \[5.4\] provides a brief conclusion of the present study.
Table 5.1: Participant demographic and clinical characteristics

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>PD subjects (mean ± std)</th>
<th>Healthy controls (mean ± std)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>60.0 ± 9.8</td>
<td>58.3 ± 7.5</td>
</tr>
<tr>
<td>Gender</td>
<td>30 females, 39 males</td>
<td>13 females, 16 males</td>
</tr>
<tr>
<td>UPDRS motor score</td>
<td>37.2 ± 17.3</td>
<td>Not applicable</td>
</tr>
<tr>
<td>Disease duration</td>
<td>6.8 ± 4.6</td>
<td>Not applicable</td>
</tr>
<tr>
<td>Hoehn and Yahr stage</td>
<td>2.3 ± 0.8</td>
<td>Not applicable</td>
</tr>
</tbody>
</table>

5.2 Methods

5.2.1 Subjects and Data Acquisition

In total, 69 PD subjects and 29 age-matched healthy controls participated in this study. The PD subjects had mild to moderate PD (Hoehn and Yahr stage I - III). All healthy controls have no history of neurological disorders. Demographic and clinical information are listed in Table 5.1. All participants were recruited from the Movement Disorders Clinic of Xuanwu Hospital of Capital Medical University, and provided written, informed consent prior to participation. All studies were approved by the Institutional Review Board of Xuanwu Hospital of Capital Medical University, Beijing, China.

Resting-state data were collected on a SIEMENS Trio 3T scanner equipped with a 16-channel head coil. During the scanning, all the participants were instructed to be awake with eyes closed and earplugs were used to minimize the machine noise. PD subjects were scanned at the off-medication state (after a 12-hour period of medication withdrawal).

High-resolution T1 weighted anatomical images were acquired using a sagittal magnetization prepared rapid gradient echo three-dimensional T1-weighted sequence with repetition time of 1970 ms, echo time of 3.9 ms, inversion time of 1100 ms and flip angle of 15°. A radiologist assessed the images for all the participants to exclude those with space-occupying lesions and cerebrovascular diseases. BOLD contrast EPI T2*-weighted images were acquired with the following specifications: repetition time of 2000 ms, echo time of 30 ms, flip angle of 90°, field of view of 256 mm × 256 mm, matrix size of 64 × 64, voxel size of 3.0 mm × 3.0 mm × 4.0 mm, axial slices of 33 layers and the scanning time of 8 mins.
The fMRI preprocessing steps were the same as those in Section 2.2.4. 76 ROIs defined by Desikan-Killiany Atlas were selected in this study as shown in Table 5.2.

Table 5.2: The 76 ROIs used in this study

<table>
<thead>
<tr>
<th>Index</th>
<th>Name</th>
<th>Index</th>
<th>Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>L1/R1</td>
<td>Left/Right-Cerebellum-Cortex</td>
<td>L20/R20</td>
<td>ctx-lh/rh-middletemporal</td>
</tr>
<tr>
<td>L2/R2</td>
<td>Left/Right-Thalamus-Proper</td>
<td>L21/R21</td>
<td>ctx-lh/rh-parahippocampal</td>
</tr>
<tr>
<td>L3/R3</td>
<td>Left/Right-Caudate</td>
<td>L22/R22</td>
<td>ctx-lh/rh-paracentral</td>
</tr>
<tr>
<td>L4/R4</td>
<td>Left/Right-Putamen</td>
<td>L23/R23</td>
<td>ctx-lh/rh-parsopercularis</td>
</tr>
<tr>
<td>L5/R5</td>
<td>Left/Right-Pallidum</td>
<td>L24/R24</td>
<td>ctx-lh/rh-parorsbitalis</td>
</tr>
<tr>
<td>L7/R7</td>
<td>Left/Right-Amygdala</td>
<td>L26/R26</td>
<td>ctx-lh/rh-pericalcarine</td>
</tr>
<tr>
<td>L8/R8</td>
<td>Left/Right-Accumbens-area</td>
<td>L27/R27</td>
<td>ctx-lh/rh-postcentral</td>
</tr>
<tr>
<td>L10/R10</td>
<td>ctx-lh/rh-caudalmiddlefrontal</td>
<td>L29/R29</td>
<td>ctx-lh/rh-precentral</td>
</tr>
<tr>
<td>L12/R12</td>
<td>ctx-lh/rh-entorhinal</td>
<td>L31/R31</td>
<td>ctx-lh/rh-rostralanteriorcingulate</td>
</tr>
<tr>
<td>L14/R14</td>
<td>ctx-lh/rh-inferiorparietal</td>
<td>L33/R33</td>
<td>ctx-lh/rh-superiorparietal</td>
</tr>
<tr>
<td>L15/R15</td>
<td>ctx-lh/rh-inferiortemporal</td>
<td>L34/R34</td>
<td>ctx-lh/rh-superiorparietal</td>
</tr>
<tr>
<td>L16/R16</td>
<td>ctx-lh/rh-lateralloccipal</td>
<td>L35/R35</td>
<td>ctx-lh/rh-superiortemporal</td>
</tr>
<tr>
<td>L17/R17</td>
<td>ctx-lh/rh-lateralorbitofrontal</td>
<td>L36/R36</td>
<td>ctx-lh/rh-supramarginal</td>
</tr>
<tr>
<td>L18/R18</td>
<td>ctx-lh/rh-lingual</td>
<td>L37/R37</td>
<td>ctx-lh/rh-transversetemporal</td>
</tr>
<tr>
<td>L19/R19</td>
<td>ctx-lh/rh-medialorbitofrontal</td>
<td>L38/R38</td>
<td>ctx-lh/rh-insula</td>
</tr>
</tbody>
</table>

“L” represents Left and “R” represents Right.

5.2.2 Dynamic Functional Connectivity Estimation

We used a sliding window approach to estimate the dynamic functional connectivity, where the sparse inverse covariance matrix was computed within each time window.
Sliding Window Analysis

Dynamic functional connectivity analysis was performed for each subject with a sliding window approach. The sliding window approach is parameterized by a window length $L$ and a step size $s$. It decomposes the entire ROI time courses into multiple overlapping temporal windows of length $L$. Specifically, given an entire ROI time courses with $N$ time points, $W = (N - L)/s + 1$ is the number of temporal windows that can be generated by applying the sliding window approach. The functional connectivity metric is then calculated within each temporal window. In this study, we choose the window length $L = 30$ TRs (60s) and the step size $s = 2$ TRs as previously suggested [136].

Sparse Inverse Covariance Matrix

As introduced in Section 1.3, the inverse covariance matrix (precision matrix) can be employed to infer brain functional connectivity. Under the assumed sparse nature of brain functional connectivity networks, a regularization strategy, such as LASSO, can be applied to the inverse covariance matrix. Functional connectivity is then indicated by the non-zero elements of the sparse inverse covariance matrix. This method is expected to be particularly useful when the number of the observations is limited, such as the limited number of time points in short temporal windows obtained from a sliding window approach.

A sparse estimate of the inverse covariance matrix is obtained by minimizing the penalized negative log likelihood

$$
\hat{\Theta} = \arg \min \{ tr(S\Theta) - \log |\Theta| + \lambda \|\Theta\|_1 \} \quad (5.1)
$$

where $\Theta$ is the inverse covariance matrix, $S$ is the sample covariance matrix, $\|\Theta\|_1$ is the element-wise L1-norm of $\Theta$, and $\lambda$ is the penalty parameter controlling the sparsity of the network. An efficient algorithm has been found to solve this optimization problem [64]. We implemented this sparse inverse covariance matrix estimation via the L1precision code [2].

5.2.3 Graph Theoretical Analysis

Graph theoretical analysis was performed on the estimated dynamic functional connectivity networks. Graph measures, including characteristic path length, global efficiency, clustering

\[\text{https://www.cs.ubc.ca/ schmidtm/Software/L1precision.html}\]
coefficient, modularity and assortativity coefficient were calculated using the brain functional connectivity toolbox [142]. Additionally, we investigated two graph spectral measures that we hypothesized would be affected by PD (described below) but have received little attention in previous studies. A list of graph measures used in this study are described in Table 5.3.

Table 5.3: Description on graph measures used in this study

<table>
<thead>
<tr>
<th>Graph Measures</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Characteristic path length</td>
<td>The average of the shortest path lengths over all pairs of nodes in a graph.</td>
</tr>
<tr>
<td>Global efficiency</td>
<td>The average inverse shortest path length of a graph.</td>
</tr>
<tr>
<td>Clustering coefficient</td>
<td>The number of connections existing among the neighbours of a node, divided by all possible connections (Average over all nodes to get an overall clustering coefficient of a graph).</td>
</tr>
<tr>
<td>Modularity</td>
<td>A statistic that quantifies the degree to which the network can be subdivided into individual groups (modules).</td>
</tr>
<tr>
<td>Assortativity</td>
<td>The correlation coefficient between the degrees of connected nodes.</td>
</tr>
<tr>
<td>Fiedler value</td>
<td>The second smallest eigenvalue of the Laplacian matrix of a graph.</td>
</tr>
<tr>
<td>Normalized Fiedler value</td>
<td>The second smallest eigenvalue of the normalized Laplacian matrix of a graph.</td>
</tr>
</tbody>
</table>

The Fiedler value [59], also known as algebraic connectivity, is an indicator of global integration of a network. The Fiedler value is computed as the second smallest eigenvalue of the Laplacian matrix of a graph $G$. The Laplacian matrix $L(G)$ is defined as [60]:

$$L(G) = D(G) - A(G)$$  \hspace{1cm} (5.2)

where $G$ is a graph with $n$ nodes, $D(G)$ is the degree matrix, an $n$-by-$n$ diagonal matrix with the elements of the diagonal equal to the degrees of the nodes in the graph $G$, and $A(G)$ is the adjacency matrix, i.e., the connectivity matrix in this study.

The other metric is the normalized Fiedler value, which is similar to the Fiedler value but normalized for the number of edges in a graph. The normalized Fiedler value is computed as the second smallest eigenvalue of the normalized Laplacian matrix. Given the $ij$th entry of the
Laplacian matrix $L_{ij}$, and the degree of the $i$th node $d_i$, the $ij$th element of the normalized Laplacian matrix $L^n_{ij}$ is calculated as $L_{ij}/\sqrt{d_i d_j}$.

### 5.2.4 Statistical Analysis

To examine whether the dynamic graph properties were different between HC and PD groups, statistical analyses were performed in each of the aforementioned graph measures using the Wilcoxon rank sum test. For each subject, time-varying functional connectivity networks were first assessed with a sliding window approach, wherein the sparse inverse covariance matrix was estimated within each temporal window. Graph measures were then calculated on the established temporal brain functional connectivity networks to get a series of dynamic graph measures. Finally, the Wilcoxon rank sum test was conducted on the standard deviations of dynamic graph measures between HCs and PD subjects.

In addition, an autoregressive (AR) model was employed to assess the dynamics in the graph measures. The AR model is a time series model that uses the observations from previous time steps to predict the value at the next step

$$y(t) = c + \sum_{i=1}^{p} a_i y(t - i) + e(t) \tag{5.3}$$

where $y$ is a time series vector, $p$ is the order of the model, $a_1, a_2, \ldots, a_p$ are the parameters of the model, $c$ is a constant, and $e(t)$ is the white noise.

Using the AR model, we examined the predictability of dynamic graph measures over time. The fitness of the AR model was assessed by the $R^2$ coefficient of determination and compared between HC and PD groups using the Wilcoxon rank sum test.

Finally, CCA was employed to investigate the relationship between dynamic graph measures and clinical features derived from PD subjects. When applying CCA to this study, dynamic graph measures and clinical information are the two sets of variables, that is, $x = [x_1, x_2, \ldots, x_m]^T$, $y = [y_1, y_2, \ldots, y_n]^T$, where $x_i (i = 1, 2, \ldots, m (= 7))$ corresponds to the standard deviation of each dynamic graph measure incorporated in the study, and $y_j (j = 1, 2, \ldots, n (= 5))$ corresponds to each clinical information shown in the Table 5.1. We then interrogated the loadings on dynamic graph measures and clinical information, which were defined respectively as the correlations between each dynamic graph measure $x_i$ and the canonical variable $X$, and between each clinical information $y_j$ and the canonical variable $Y$, to interpret
the relationship between dynamic graph measures and clinical information.

5.2.5 Disease Classification

To evaluate the predictive ability of dynamic graph properties for disease identification, we compared classification performance between HCs and PD subjects using different features. A feature vector, with a large amount of features, is generated based on these dynamic time series of graph measures. With the high dimensionality of feature space, the complexity of the model used for classification is dramatically increased and redundancy in features degrades classification performance. In order to avoid the effect of curse of dimensionality, feature extraction was first performed by calculating 4 statistical indices, including the mean value, standard deviation, minimum and maximum value, to characterize the time series of each graph measure, which led to a reduced feature dimension of 28 (4 statistics \( \times \) 7 graph measures), and then feature selection was applied to the reduced feature set to obtain the most discriminative ones as the final feature set. Here a filter feature selection algorithm, i.e., the ReliefF algorithm [101], was used to sort the features based on their individual discriminative ability.

Different supervised classifiers, including random forest, support vector machine (SVM), logistic regression, linear discriminant analysis (LDA) and naïve Bayes, were used to examine the discriminative power of dynamic graph measures. The random forest classifier was composed of 100 trees, and the SVM classifier was implemented with a radial base function (RBF) kernel. The same ten-fold cross validation strategy was performed for each of the five classifiers to evaluate classification performance. For each training set, we performed feature selection on the training dataset and the classifier was trained using the selected features. We then applied the trained classifier to the testing dataset with the selected features to estimate classification performance. Final classification performance was obtained by averaging over all folds.

In addition, to investigate the role of different dynamic graph measures in disease classification, we compared classification performances under different scenarios: with and without each dynamic graph measure. Furthermore, we compared classification performance using stationary graph measures (assessed by the mean value of temporal graph measures), dynamic graph measures (assessed by the standard deviation, minimum and maximum value of temporal graph measures), and a combination of stationary and dynamic graph measures.

The overall framework proposed in this chapter is graphically shown in Figure 5.1. Given the extracted time series of each ROI, time-varying functional connectivity networks were es-
estimated using the sliding window approach. Graph measures were then calculated on each temporal network to generate a series of dynamic graph measures. Feature extraction and feature selection were performed prior to proceeding to the classification step. Finally, different classifiers were applied to evaluate the classification performance.

5.3 Experimental Results and Discussion

5.3.1 Dynamic Graph Theoretical Analysis

One example of the functional connectivity network at one time point is graphically shown in Figure 5.2. Graph theoretical analysis was applied to temporal functional connectivity networks to obtain a series of graph measures over time, quantitatively describing the dynamic topological properties. In order to characterize the patterns of dynamic graph measures, the standard deviation of each dynamic graph measure was calculated and compared between the two groups. The Wilcoxon rank sum test was performed on the standard deviations to determine if the changing patterns of dynamic graph measures were statistically different between HCs and PD subjects. The dynamic graph measures with significant differences between HC and PD groups are shown in Figure 5.3. PD subjects exhibited lower standard deviation in the Fiedler value ($p = 0.003$, FDR (False Discovery Rate) corrected) and modularity ($p = 0.04$, FDR corrected),
suggesting both the global integration and local segregation of brain networks were less variable in PD. The standard deviation of the global efficiency was also found to be moderately smaller in PD subjects ($p = 0.07$, FDR corrected), suggesting a potential lack of variability in the parallel information transfer in brain networks of PD.

In addition to the standard deviation of dynamic graph measures, an AR model was also employed to explore the dynamics of the graph measures. Here a second order AR model was fitted on each graph measure, and the $R^2$ coefficient of determination was calculated and compared between HCs and PD subjects. Significant differences were found in the Fiedler value ($p = 0.03$, FDR corrected), characteristic path length ($p = 0.03$, FDR corrected), global efficiency ($p = 0.03$, FDR corrected) and modularity ($p = 0.03$, FDR corrected) with the Wilcoxon rank sum test, as shown in Figure 5.4. The higher coefficient of determination indicated the dynamic Fiedler value, characteristic path length, global efficiency and modularity were more predictable over time in HCs, while PD subjects tended to exhibit a less deterministic dynamic pattern.

Finally, CCA was performed on the dynamic graph measures and clinical information to determine the relationship between them. Figure 5.5 shows the loadings (both positive and negative) on dynamic graph measures and clinical information. One significant CCA component
Figure 5.3: The comparison of standard deviations between HC and PD groups with respect to the Fiedler value, modularity and global efficiency.

(i.e., the first pair of canonical variables) was found ($p < 0.05$). Specifically, the dynamic Fiedler value (its counterpart) and modularity loaded highly among all dynamic graph measures, and the H&Y score (a measure of overall disease severity) loaded highest among the clinical information, suggesting a relationship between disease severity and the dynamics in the global integration and local segregation of brain networks.

5.3.2 Classification Performance

Although the statistical analysis results revealed group differences in the dynamic graph properties between HCs and PD subjects, such group level differences have reduced applicability in a clinical setting, where a premium is placed on making inferences at an individual level. To further examine the utility of dynamic graph measures as features for automatic diagnosis of PD, we employed the classification technique to differentiate HCs and PD subjects based on their dynamic graph measures. In particular, we are interested in the role of dynamic graph measures, and especially, the Fiedler value for PD identification.
The classification performances across different classifiers are shown in Table 5.4. Two sets of experiments were done to examine the discriminative power of dynamic graph measures and the role of the Fiedler value among all graph measures. In the first experiment, we first fed all other graph measures, including characteristic path length, global efficiency, clustering coefficient, modularity and assortativity (in total, 4 statistics \(\times\) 5 graph measures = 20 features), into the classifiers to obtain the classification performance as shown in the first row of Table 5.4. Then the Fiedler value and its counterpart (i.e., normalized Fiedler value) were added to the above graph measures (in total, 4 statistics \(\times\) 7 graph measures = 28 features) and fed into the classifiers to examine the effect of the Fiedler value. The corresponding classification performance are shown in the second row of Table 5.4. In the second experiment, we applied the ReliefF feature selection method to the settings of the first experiment. In each case, we selected the top 5 features as the input of the classifiers. We first applied feature selection to all other graph measures without the Fiedler value to obtain the classification performance as shown in the third row of Table 5.4, and then applied feature selection to all other graph measures with the Fiedler value to obtain the classification performance as shown in the last row of Table 5.4. As can be seen from Table 5.4, in both cases (before and after feature selection), while all other graph measures weakly differentiated HCs and PD subjects, the incorporation of the Fiedler
value significantly improved classification performance. When applying feature selection to all 7 graph measures (the classification results in the last row of Table 5.4), the top 5 features corresponded to the statistics of the Fiedler value and normalized Fiedler value, suggesting the Fiedler value played a more important role than other graph measures in disease classification. The top features among all other graph measures (i.e., characteristic path length, global efficiency, clustering coefficient, modularity and assortativity) corresponded to the statistics of modularity and global efficiency, which also showed different levels of significance in the statistical analysis. A best classification performance of 85.7% was achieved using random forest, suggesting the utility of dynamic graph measures as features for PD identification.

The effect of each dynamic graph measure on the classification performance is graphically shown in Figure 5.6. The classification performance differences were minor for all other dynamic
graph measures. However, a significant improvement on the classification performance was observed by the inclusion of dynamic Fiedler value, supporting the important role of Fiedler value for disease identification.

Classification performance using stationary graph measures, dynamic graph measures and a combination of stationary and dynamic graph measures were compared, as shown in Figure 5.7. Inclusion of dynamic graph measures is important for the development of MRI-derived biomarkers for PD diagnosis.

We compared our results with the current state-of-the-art in Table 5.5. The classification performance of our proposed work is comparable with previous studies in literature. Although the performance is not among the best, we still consider it satisfactory considering the dramatic reduction in the number of features used for disease identification, and the larger sample size utilized here.

5.3.3 Discussion

In this chapter, we investigated the graph properties of time-varying functional connectivity in HCs and PD subjects derived from resting-state fMRI data. Dynamic functional connectivity networks were estimated by the sparse inverse covariance matrix across sliding windows, and graph theoretical analysis was performed on the established time-varying functional connectivity networks to develop non-invasive biomarkers. The computed dynamic graph measures were fed into the classifier to evaluate their utility for automatic disease detection. All findings demonstrated the importance of including dynamic graph properties, especially fluctuations in
Table 5.5: Comparison between the proposed work and the state-of-the-art in PD classification.

<table>
<thead>
<tr>
<th>Work</th>
<th>Subjects</th>
<th>Modality</th>
<th>Features</th>
<th>#</th>
<th>Classifier</th>
<th>Acc</th>
</tr>
</thead>
<tbody>
<tr>
<td>[46]</td>
<td>PD21/HC26</td>
<td>fMRI</td>
<td>Functional connectivity</td>
<td>150</td>
<td>SVM</td>
<td>93.6%</td>
</tr>
<tr>
<td>[103]</td>
<td>PD19/HC27</td>
<td>fMRI and sMRI</td>
<td>ALFF, ReHo, RFCS, GM, WM, CSF</td>
<td>40</td>
<td>SVM</td>
<td>86.9%</td>
</tr>
<tr>
<td>[137]</td>
<td>PD30/HC30</td>
<td>sMRI</td>
<td>Values of voxels</td>
<td>417</td>
<td>SVM</td>
<td>86.7%</td>
</tr>
<tr>
<td>[95]</td>
<td>PD123/HC85</td>
<td>sMRI and DTI</td>
<td>GM, CSF and FA</td>
<td>10</td>
<td>SVM</td>
<td>83.3%</td>
</tr>
<tr>
<td>[147]</td>
<td>PD50/HC50</td>
<td>DTI</td>
<td>FA and MD</td>
<td>14</td>
<td>Logistic Regression</td>
<td>77.2%</td>
</tr>
<tr>
<td>Ours</td>
<td>PD69/HC29</td>
<td>fMRI</td>
<td>Dynamic graph measures</td>
<td>5</td>
<td>Random Forest</td>
<td>85.7%</td>
</tr>
</tbody>
</table>

fMRI = functional MRI; sMRI = structural MRI; DTI = Diffusion Tensor Imaging; ALFF = Amplitude of Low-frequency Fluctuations; ReHo = Regional Homogeneity; RFCS = Regional Functional Connectivity Strength; GM = Gray Matter; WM = White Matter; CSF = Cerebrospinal Fluid; FA = Fractional Anisotropy; MD = Mean Diffusivity.
the Fiedler value, for the development of an MRI-related biomarker for PD.

Graph theoretical analysis has been increasingly employed to study brain networks in a variety of neurological diseases, revealing significant altered graph properties in diseased brains [14, 170]. However, unlike the large number of graph theoretical analyses in diseases such as AD, limited studies have investigated graph properties in PD [3, 131]. Existing studies, utilizing static connectivity measures, suggested that topological properties of functional connectivity networks were altered in PD, with a decreased global efficiency and increased clustering coefficient and modularity compared to HCs [10, 159]. In addition, another study investigated topological properties of structural brain networks in PD, demonstrating aberrant cerebral network topology with a larger characteristic path length and reduced global efficiency in comparison with HCs [131].

The human brain is obviously a dynamically interactive system, and even at the relatively sluggish temporal resolution of fMRI, changes in connectivity patterns are evident [4, 142]. Recent studies have examined the altered dynamics of functional connectivity in disease states [84, 145]. However, relatively few studies have explored dynamic graph measures of time-varying functional connectivity networks (e.g., [89, 202]). In the current study, we analyzed dynamic graph properties in PD using fMRI data. In a study that compared the graph measures with
different network densities, the values of graph measures were found to be dependent on the network sparsity [25]. A low density without isolated nodes within the network was suggested in graph theoretical analysis. We used a sparse inverse covariance matrix in our study. In this chapter, a fixed parameter with sparsity level as 20% was chosen. We found significant differences between HCs and PD subjects in terms of the Fiedler value and modularity, suggesting abnormal dynamics in both global integration and local segregation of diseased brain networks. Further CCA analysis revealed such dynamics were closely related to disease severity. In addition to the standard deviation which was mostly used to analyze dynamic patterns of features, an autoregressive model was further employed in this study to investigate the predictability of dynamic graph measures over time. PD subjects had lower $R^2$ of the model (indicating less predictable) in dynamic Fiedler value, characteristic path length, global efficiency and modularity, suggesting less deterministic dynamics in disease states. In the current work, we included the Fiedler value in the graph theoretical analysis, which has received little attention in previous studies. We found PD subjects had an especially lower variability in dynamic Fiedler value of brain networks. This is suggestive of a reduction in the variability of global integration of diseased brain networks, possibly due to the impaired cognitive flexibility in PD. Two prior studies have applied the Fiedler value to studying structural connectivity network properties in patients with AD [51, 134]. One study revealed a decreased Fiedler value in patients, suggesting a reduced network robustness as disease progressed, whereas the other study showed few
differences in the Fiedler value across diagnostic groups. In contrast, the current study applied the Fiedler value to time-varying functional connectivity networks, showing for the first time disrupted temporal dynamics in global integration of brain networks in PD and its relation to severity of the disease.

In addition to the statistical group-wise analysis between HCs and PD subjects, we utilized machine learning techniques to make individual-based inferences using dynamic graph measures. There are several recent studies making use of graph measures for the diagnosis of neurological diseases. For example, Khazaee et al. [87] used graph measures to identify patients with AD and mild cognitive impairment (MCI) from healthy controls. However, disease identification studies using dynamic graph properties remain scarce. In [189], Wee et al. employed, for the first time, dynamic graph measure (i.e., clustering coefficient) for early MCI identification. With respect to PD, previous studies looking at MRI classification of PD have relied upon static features, such as structural/functional connectivity, ALFF or ReHo, with the accuracy ranging from 39.53% to 93.6% [46, 103, 137]. To the best of our knowledge, this is the first report to explore the utility of dynamic graph measures for PD classification. A classification accuracy of 85.7% suggested good diagnostic power of dynamic graph measures. Although such classification performance is not the highest in the literature, we can still consider it satisfactory given the very low number of features used, and the relatively large sample size employed. The classification performance of the current work is higher than that of the early MCI identification study using temporal clustering coefficients which yielded 79.6% classification accuracy [189]. Promising classification results suggested that inclusion of dynamic graph measures, and in particular, the Fiedler value, will be important for the development of MRI-related biomarkers for PD.

5.3.4 Limitations

We have performed dynamic graph theoretical analysis of time-varying functional connectivity networks in PD, revealing the altered dynamic graph properties in diseased brains. However, the underlying neurobiological underpinnings for such dynamics remain unclear. Future studies will be required to advance our understanding of these temporal dynamics. In addition, the dynamic graph measures were computed on time-varying functional connectivity networks which were estimated by the sparse inverse covariance matrix across sliding windows. However, we cannot rule out the possibility that different graph creation methods may give varied results. As
suggested in [134], there has been little consistency in previous studies with respect to graph theoretical analysis even though using the same image modality, in part due to the different graph creation methods. Therefore, future comprehensive studies using a wide range of graph creation methods should be performed to verify the reliability of dynamic graph measures as biomarkers for PD.

5.4 Conclusion

In this chapter, we analyzed dynamic graph properties of time-varying functional brain functional connectivity in HCs and PD subjects. In particular, the Fiedler value, a novel graph spectral measure, was explored for studying the dynamics of functional connectivity. PD subjects had an altered variability in dynamic Fiedler value and modularity of brain networks, which was related to disease severity. The dynamics in Fiedler value, along with characteristic path length, global efficiency and modularity, were less deterministic in PD. Promising classification results provided support for including dynamic graph measures, especially dynamic Fiedler value, for MRI assessment of PD.
Chapter 6

Conclusion and Future Work

6.1 Conclusion

Inferring brain functional connectivity from fMRI data can take place at the voxel or ROI level. In this thesis, we focus on the ROI-based brain functional connectivity study. From the application perspective, brain functional connectivity assessment can be broadly divided into two major categories: brainstem functional connectivity and cortical/subcortical brain functional connectivity. While the former is rarely studied and the latter is commonly studied, we investigate both of them in this thesis (for brainstem functional connectivity, we specifically focus on brainstem-cortical functional connectivity). From the methodological perspective, we address fundamental problems associated with ROI-based brain functional connectivity study, including regional signal representation, brain functional connectivity modelling and brain functional connectivity analysis. All the proposed methods have been applied to real fMRI data. The results revealed reliable brain functional connectivity estimation and/or indicated successful disease assessment and treatment evaluation. A summary of the thesis contributions and major findings are listed below.

First, we proposed a novel framework for brainstem-cortical functional connectivity modelling where the regional signal of brainstem nuclei is appropriately represented by PLS and the connections between brainstem nuclei and other cortical/subcortical brain regions are reliably estimated by partial correlation. Since brainstem structures can be highly functionally inhomogeneous, it may not be optimal to use average signal based regional signal representation strategy. PLS is a reasonable alternative approach that represents regional signals by utilizing the covariance relationship between brainstem nuclei and other cortical/subcortical brain regions. Additionally, considering the anatomical peculiarity of the brainstem, in our proposed framework, special care is taken in the preprocessing wherein a separate brainstem motion correction is performed. We applied the proposed framework to assess functional connectivity of the PPN, a brainstem nucleus critical for locomotion control. Specifically, in Chapter
2 and 3, we employed the proposed framework to examine the effect of two clinical interventions, namely GVS and walking exercise, on PPN functional connectivity. Our method reliably assisted in the treatment evaluation of PD in terms of brainstem-cortical functional connectivity. The results demonstrated that PPN functional connectivity can be modulated by GVS in a stimulus-dependent manner as well as by walking exercise in a dose-dependent manner in PD, which may guide how these clinical interventions can induce functional plasticity and thus provide insights into their underlying mechanism of disease treatment.

Second, in Chapter 4, we proposed a novel method of representing regional signal and estimating brain functional connectivity with constrained canonical correlation analysis. With most ROI-based brain functional connectivity modelling approaches, the signals from same-ROI voxels are simply averaged, neglecting any inhomogeneity in each ROI. Our proposed method is able to take the regionally-specific nature of brain activity, the spatial location of concentrated activity, and activity in other ROIs into account for simultaneous regional signal representation and brain functional connectivity estimation. Using the proposed method, we estimated brain functional connectivity between subcortical brain regions in healthy adults. Our results demonstrated that the proposed method is a robust model for assessing brain functional connectivity, yielding a more reproducible connectivity pattern.

Finally, given the inferred brain functional connectivity network, further analysis to summarize network properties is of great importance, especially for dynamic brain functional connectivity which contains rich spatiotemporal information. In Chapter 5, we proposed the use of graph theory in a dynamic manner to extract useful information from brain functional connectivity network for the development of MRI-related biomarkers. In particular, a novel graph spectral metric, Fiedler value, was introduced for studying the dynamics of functional connectivity. When applied to studying brain dynamics in PD, we found altered dynamic graph properties, including Fiedler value, in PD compared to healthy controls. In addition, we obtained promising classification performance using dynamic graph properties, and indicated that Fiedler value was the most important feature for improving the diagnosis power. Our findings demonstrated the importance of including dynamic graph properties, especially fluctuations in the Fiedler value, for the development of an MRI-related biomarker for PD.
6.2 Future Work

6.2.1 Multi-task Brainstem-cortical Functional Connectivity Analysis

In this thesis, we have proposed a framework for analyzing brainstem-cortical functional connectivity under one condition (e.g., resting-state or under stimuli). In clinical applications, the experiment design could incorporate multiple tasks and it would be of interest to investigate the differential effects of tasks. Therefore, we would like to extend the one-condition brainstem-cortical functional connectivity analysis to multi-task brainstem-cortical functional connectivity analysis.

Discriminant correlation analysis (DCA) \[73\] is a recently proposed approach for analyzing the correlation relationship between two datasets. Similar to the CCA method, it maximizes the correlation between two datasets, while the DCA method also incorporates the class associations, wherein the between-class correlations within each dataset are eliminated and the correlation relationships are restricted to be within classes. Therefore, DCA is well suited for multi-task connectivity analysis, wherein each task is considered as one class. We are interested in applying the DCA method to investigate the multi-task brainstem-cortical functional connectivity. By applying DCA, the two datasets correspond to the time courses of cortical/subcortical ROIs and the time courses of a brainstem area, and in each dataset, time courses corresponding to different tasks are incorporated.

6.2.2 Regional Signal Representation with Constrained Multiset Canonical Correlation Analysis for Brain Functional Connectivity Network Estimation

In ROI-based brain functional connectivity estimation, a key issue is how to represent the information in a given ROI. Previous studies have suggested that different regional signal representation strategies may have a significant impact on the subsequent analysis inferred from the fMRI data. Most current studies take the average signal from same-ROI voxels for regional signal representation. Alternative strategies such as PCA-based approach have been proposed in the literature. In Chapter 4, we propose a novel regional signal representation method using constrained CCA and demonstrate its superiority over average-signal and PCA based approaches in terms of the accuracy of the estimated brain functional connectivity and the reproducibility of the connectivity pattern. However, our proposed method is a pairwise
regional signal representation strategy which is conducted between pairs of ROIs. To facilitate the computational speed and the large-scale analysis, it would be desirable to develop a joint regional signal representation method which could simultaneously assess representative signals across all ROIs.

Multiset CCA (MCCA) [98] is a statistical method extended from the CCA to summarize the correlation structure among different datasets. While CCA maximizes the correlation relationships between two datasets via linear transformation, MCCA extends the CCA to multiple datasets in which the overall correlation among multiple (i.e., more than two) datasets is optimized. By applying MCCA, the corresponding canonical variables from each dataset can be jointly obtained.

Inspired by the potential of MCCA, we are interested in extending the pairwise to joint regional signal representation for brain functional connectivity estimation. Similar to the pairwise regional signal representation with constrained CCA, spatial constraints will be incorporated into the MCCA for the joint regional signal representation. Given a set of ROIs, instead of performing constrained CCA on each pair of ROIs, MCCA will be carried out on all ROIs to jointly obtain the regional representative signals at one time. Meanwhile, the correlation matrix of representative signals will be available for brain functional connectivity estimation.

### 6.2.3 Deep Clustering for Automated Brain Parcellation

Brain parcellation refers to the division of the brain into a set of regions (or parcels) that share certain neurobiological characteristics. It is an active research area which provides insights into the underlying brain architecture. Appropriate brain parcellation is always a prerequisite and of great significance for the subsequent quantitative analysis of the brain [19]. While manual parcellation of the brain is laborious, subjective and time-consuming, automated brain parcellation is highly desired in practice for quantitative assessment.

Many approaches have been proposed for the automated parcellation of the brain. Conventional strategies can be categorized into: (1) atlas-based registration, (2) data-driven methods such as ICA and clustering algorithms, and (3) machine learning with hand-crafted features. However, these conventional methods suffer from disadvantages including potential registration errors, dependence on manual feature engineering and shallow structures with limited power, especially for sophisticated high-dimensional features. Recently, with the emergence of deep learning, researchers have leveraged this technique to perform brain parcellation, accounting for
the shortcomings of previous methods. Although significant improvements have been achieved via deep learning, this technique highly depends on the labelled training data for supervised learning. Nevertheless, in the case of lacking labelled data and exploratory studies where the underlying ground truth is not explicitly clear, we would be more interested in unsupervised deep learning.

Most recently, efforts have been dedicated to adapting clustering algorithms to end-to-end learning using the deep neural network, which we call Deep Clustering. It simultaneously learns feature representations and cluster assignments in an unsupervised manner. Different algorithms have been developed, including Deep Embedded Clustering (DEC) [196], Deep Embedded Regularized Clustering (DEPICT) [70], and DeepCluster [34], showing significant improvements over traditional clustering algorithms. While deep clustering is a recently emerging method, its application to the medical area remains scarce. In the future work, we plan to apply deep clustering to brain parcellation. Specifically, we would like to conduct a voxel-wise brain parcellation on the basis of brain functional connectivity, under the assumption that the patterns of functional connectivity are of high dimension with complexly organized hierarchical features that could be extracted by deep neural network and used for partitioning the brain into distinct functional regions. An interesting application would be to investigate functional regions contained in the brainstem, which could provide insights into the underlying functional structures of the brainstem and ultimately benefit clinical neuroscience.

6.2.4 Application to Parkinson’s Disease Studies

As one of the most common degenerative neurological disorders, Parkinson’s disease affects a large population worldwide, particularly in people over 50 years of age. The relatively high prevalence and serious consequences of PD have a significant impact on global health. While traditionally the diagnosis of PD has been clinical, and traditional structural imaging methods have been relatively uninformative, fMRI may prove diagnostically and prognostically useful for PD.

It has been suggested that neurological disorders including PD can be characterized as dysconnection syndromes. The study of brain functional connectivity from fMRI data could provide insights into the disease related connectivity abnormality, which may ultimately assist in the disease diagnosis and treatment assessment.

In future work, we plan to investigate brain functional connectivity in PD, examining the
disease induced effect on connectivity patterns. In particular, we are interested in studying the brainstem functional connectivity in PD. It would be interesting to examine functional connections of different brainstem nucleus, evaluate the effects of various therapeutic interventions on brainstem functional connectivity and characterize functional organizations of the brainstem.
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