APPROACHES TO EXTRACT, CHARACTERIZE, AND INTERPRET DYNAMIC FUNCTIONAL NETWORK CONNECTIVITY IN FMRI DATA

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To my family!
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Figure 8-1: **Analytic pipeline.** **Step1:** The time-course signal of seven regions in the default mode network (DMN) has been identified using group-ICA. **Step2:** After identifying seven regions in DMN, a taper sliding window was used to segment the time-course signals and then calculated the FNC matrix. Each FNC matrix contains twenty-one connectivity features. Each feature represents the connectivity between any pair of DMN subnodes. **Step3:** After vectorizing the FNC matrixes, we concatenated them, and then a k-means clustering was used to group FNCs into five distinct clusters. Then, hidden Markov model (HMM) features, in total of 25 features, were calculated from the state vector of
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**Figure 8-2: Feature selection.** The connectivity features of seven default mode network (DMN) subnodes were used as input to fit logistic regression as a classifier to discriminate SZ from HC. With seven subnodes of DMN, we had twenty-one connectivity features. Elastic net regularization (ENR), as a feature selection, used the model generated by the classifier and the input features to find the feature that was the most predictive in discriminating between the two classes. ACC: Anterior cingulate cortex, PCC: posterior cingulate cortex, PCu: Precuneus.

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**Figure 8-5: Feature selection results in FBIRN dataset.** The left panel shows the ROC of the classification between SZ and HC in each state. The area under ROC or AUC of the SZ vs. HC classification was significantly higher than the change in all states. The right panel shows the most important features based on elastic net regularization that had an equal and significant contribution to the classification. The colorful features are selected by multiple comparison ANOVA tests. AUC: Area under the curve.

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**Figure 10-4: Potential clinical benefit of the result.** Our results suggest a possible benefit of changing the brain state with higher ACC connectivity and lower PCu/PCC connectivity and changing that state to a state with lower ACC and higher PCu/PCC connectivity.

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### LIST OF SYMBOLS AND ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>ACC</td>
<td>anterior cingulate cortex</td>
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<tr>
<td>AD</td>
<td>Alzheimer's disease</td>
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<tr>
<td>ADASYN</td>
<td>augmentation method called adaptive synthetic</td>
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<tr>
<td>ADN</td>
<td>auditory network</td>
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<tr>
<td>ANOVA</td>
<td>analysis of variance</td>
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<td>APOE</td>
<td>Apolipoprotein E</td>
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<td>AUC</td>
<td>area under the curve</td>
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<tr>
<td>Bold</td>
<td>blood-oxygen-level-dependent</td>
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<td>CAT</td>
<td>CATBoost</td>
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<tr>
<td>CB</td>
<td>cerebellum</td>
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<tr>
<td>CBN</td>
<td>cerebellar network</td>
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<tr>
<td>CCN</td>
<td>cognitive control network</td>
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<td>CDR-SOB</td>
<td>clinical dementia rating scale sum of boxes</td>
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<td>CMINDS</td>
<td>computerized multiphasic interactive neurocognitive diagnostics system</td>
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<tr>
<td>CNN</td>
<td>convolutional neural network</td>
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<tr>
<td>COBRE</td>
<td>Center for Biomedical Research Excellence</td>
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<td>CV</td>
<td>cross validation</td>
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<td>Acronym</td>
<td>Definition</td>
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<tr>
<td>CalcarineG</td>
<td>calcarine gyrus</td>
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<td>DBS</td>
<td>deep brain stimulation</td>
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<td>DEP</td>
<td>depression</td>
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<tr>
<td>dFNC</td>
<td>dynamic functional network connectivity</td>
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<td>DMN</td>
<td>default mode network</td>
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<td>ECT</td>
<td>electroconvulsive therapy</td>
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<td>ENR</td>
<td>elastic net regularization</td>
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<td>EPI</td>
<td>echo-planar imaging</td>
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<td>FBIRN</td>
<td>Functional Imaging Biomedical Informatics Research Network</td>
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<tr>
<td>FC</td>
<td>functional connectivity</td>
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<td>FDR</td>
<td>false discovery rate</td>
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<td>FI</td>
<td>fluid intelligence</td>
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<td>FN</td>
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<td>FN</td>
<td>frontal network</td>
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<tr>
<td>FNC</td>
<td>functional network connectivity</td>
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<td>FP</td>
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<td>FWHM</td>
<td>full width at half maximum</td>
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<td>G2PC</td>
<td>global permutation percent change</td>
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HC  healthy control
HCP-YA  Human Connectome Project Young Adult
HDRS  Hamilton depression rating scale
HGR-AD  high genetic risk AD
HMM  hidden Markov model
HiPP  hippocampus
ICA  independent component analysis
ICN  intrinsic component network
IFG  inferior frontal gyrus
IOG  inferior occipital gyrus
IPL  inferior parietal lobule
LASSO  least absolute shrinkage and selection operator
LGR-AD  low genetic risk AD
LR  logistic regression
LingualG  lingual gyrus
MA  middle adult
MCC  middle cingulate cortex
MCI  mild cognitive impairment
<table>
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<tr>
<th>Abbreviation</th>
<th>Full Form</th>
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<td>MDD</td>
<td>major depressive disorder</td>
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<tr>
<td>MGR-AD</td>
<td>medium genetic risk AD</td>
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<td>MI</td>
<td>mutual information</td>
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<td>MNI</td>
<td>Montreal Neurological Institute</td>
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<td>MOG</td>
<td>middle occipital gyrus</td>
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<td>MPFC</td>
<td>medial prefrontal cortical</td>
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<td>MTG</td>
<td>middle temporal gyrus</td>
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<tr>
<td>MiFG</td>
<td>middle frontal gyrus</td>
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<tr>
<td>NOT</td>
<td>number of transitions</td>
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<tr>
<td>OA</td>
<td>old adult</td>
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<td>OASIS</td>
<td>Open Access Series of Imaging Studies</td>
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<tr>
<td>OCR</td>
<td>occupancy rate</td>
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<tr>
<td>PANSS</td>
<td>positive and negative syndrome scale</td>
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<tr>
<td>PCA</td>
<td>principal component analysis</td>
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<td>PCC</td>
<td>posterior cingulate cortex</td>
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<td>PCu</td>
<td>precuneus</td>
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<td>ParaCL</td>
<td>paracentral lobule</td>
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<td>Abbreviation</td>
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<tr>
<td>PoCG</td>
<td>postcentral gyrus</td>
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<td>PreCG</td>
<td>precentral gyrus</td>
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<tr>
<td>RF</td>
<td>random forest</td>
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<td>ROC</td>
<td>receiver operating characteristic curve</td>
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<td>RT</td>
<td>reaction time</td>
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<td>SCN</td>
<td>subcortical network</td>
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<td>SFG</td>
<td>superior frontal gyrus</td>
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<tr>
<td>sFNC</td>
<td>static functional network connectivity</td>
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<td>SHAP</td>
<td>Shapley additive explanation</td>
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<td>SICV</td>
<td>subcortical ischemic vascular disease</td>
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<td>SMA</td>
<td>supplementary motor area</td>
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<td>SMFG</td>
<td>superior medial frontal gyrus</td>
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<td>SMN</td>
<td>sensorimotor network</td>
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<td>SPL</td>
<td>superior parietal lobule</td>
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<td>SPM</td>
<td>statistical parametric mapping</td>
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<td>STG</td>
<td>superior temporal gyrus</td>
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<td>SVM</td>
<td>support vector machine</td>
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<td>SVR</td>
<td>support vector regression</td>
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<tr>
<td>SZ</td>
<td>schizophrenia</td>
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<td>TE</td>
<td>echo time</td>
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<td>TN</td>
<td>true negative</td>
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<td>TP</td>
<td>true positive</td>
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<tr>
<td>TR</td>
<td>repetition time</td>
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<td>VFLT</td>
<td>visual figure learning test</td>
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<td>VMAD</td>
<td>very mild AD</td>
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<td>VSN</td>
<td>visual sensory network</td>
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<td>VSN</td>
<td>visual network</td>
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<td>XGB</td>
<td>XGBoost</td>
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<td>fMRI</td>
<td>functional magnetic resonance imaging</td>
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<tr>
<td>fov</td>
<td>field of view</td>
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<td>rTMS</td>
<td>repetitive transcranial magnetic stimulation</td>
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SUMMARY

The objective of this project is to develop new approaches for analyzing dynamic functional network connectivity (dFNC) and investigate the link between dFNC with cognitive score and symptom severity in different neurological disorders, including schizophrenia, major depressive disorder, and Alzheimer’s disease. Knowing that the brain is highly dynamic during the resting-state fMRI, even in the absence of external inputs, dFNC got much attention in recent years. However, there are still some gaps in the field. These gaps include the lack of an analytic pipeline analyzing big dFNC data, a pipeline uncovering hidden dynamics masked by the highly influential networks, a comprehensive toolbox extracting dFNC features, and a lack of understanding of the clinical benefit of dFNC results. In this Ph.D. proposal, we aim to address these potential gaps.

This Ph.D. dissertation contributed to the field by developing new frameworks (methodological contributions) and identifying new biomarkers in brain disorders (clinical contributions). We proposed multiple frameworks to analyze both static (sFNC) and dynamic functional network connectivity (dFNC) for the former contributions. We developed a framework called iSparse k-means to analyze big dFNC data. We showed that this framework analyzes dFNC data 27 times faster than the conventional framework, but it does not need huge computational power. We also developed an analytic pipeline (toolbox), called “DyConX”, to estimate transient states and extract temporal features from dFNC. Also, we introduced some additional summary metrics to characterize dFNC. We validated these new features with the new toolbox in the largest dFNC analysis ever. Also, we introduced a new pipeline to uncover hidden dynamics of the brain network masked with highly active networks. Next, we proposed integrating our pipeline with an
interpretable machine learning method to investigate the use of dynamic features to be useful as predictors (or biomarkers).

We identified new dFNC biomarkers for the latter contributions in Alzheimer’s disease, schizophrenia, and major depressive disorder. Additionally, we interpreted how dFNC information is linked with symptom severity in these neurological and neuropsychiatric disorders.
CHAPTER 1. INTRODUCTION AND BACKGROUND

Neurological disorders comprise more than 600 conditions that impact an estimated 50 million Americans every year. This type of disease causes impairment in the functionality of the central nervous system or the peripheral nervous systems and chronic physical, cognitive, and emotional disability. Understanding the underlying mechanism of neurological disorders guides doctors in diagnosing them and provides new insight into possible treatment. To understand the neural process behind different brain diseases, we first need to measure brain activities.

Among all modalities that measure brain activity, functional magnetic resonance imaging (fMRI), which revolutionized neuroscience-related research over the past decade, provides unique information about brain alteration in the brain of healthy individuals and patients [1], [2]. It is a non-invasive imaging method that detects regional, time-varying brain metabolic changes, including blood flow and deoxygenated hemoglobin levels [3]. These metabolic changes can be induced by a cognitive task (task-based fMRI) or an unregulated brain process in the resting due to neurological disorders (resting-state fMRI).

![Figure 1-1: Static Functional network connectivity (FNC). Pearson correlation between any pair of nodes would be used to measure the combination between those nodes. We can represent all connectivity with an FNC matrix. With N nodes, we would have an N×N FNC matrix.](image)
The fMRI indirectly measures brain electrical activity based on three fundamental principles: 1) variations in the relative concentration of oxygen in the local blood supply are regularly linked to changes in brain activity, 2) in comparison to deoxygenated blood, oxygenated blood has a distinct magnetic susceptibility, 3) by assessing BOLD response, fMRI may infer changes in the ratio of oxygenated/deoxygenated blood (hemodynamic response function).

Functional connectivity (FC) or its network analog functional network connectivity (FNC), as shown in Figure 1-1, studies the correlation between the BOLD signals from different brain regions. FC/FNC, which measures the communication between brain networks, has been shown to play a key role in complex cognitive processes. It can provide insight into how large-scale neuronal communication in the human brain relates to human behavior and how this relationship may be altered in neurodegenerative disease. It has revealed a great deal of knowledge about the brain's macro-scale spatiotemporal organization in

Figure 1-2: Static (sFNC) and dynamic Functional network connectivity (dFNC). We use a sliding window to estimate dynamic FNC. Within each window, we calculate FNC across all brain regions. While sFNC is estimated from the entire signal.
healthy subjects and patients with various neurological disorders. Many of these analyses have ignored the dynamics by assuming that FNC is static over time. Indeed, functional connectivity is highly dynamic, even in the absence of external inputs. In fact, dFC/dFNC research suggests that cognitive deficits and clinical symptoms associated with many neurological disorders depend not only on the strength of the connectivity between any pair of brain regions but also on the variation of those regions' connectivity strength over time.

1.1 dFNC methodological framework

1.1.1 Sliding window approach

In this approach, we use a temporal window with the size of \( W \) and move that from the beginning to the end of the time-course signal (Figure 1-2). Using the Pearson correlation coefficient, as shown below, we measure the connectivity between any signal pair within each window.

\[
R = \frac{\sum_{n=1}^{N}(x_1 - \bar{x}_1)(x_2 - \bar{x}_2)}{\sqrt{\sum_{n=1}^{N}(x_1 - \bar{x}_1)^2 \sum_{n=1}^{N}(x_2 - \bar{x}_2)^2}}
\]

where \( x_1 \) and \( x_2 \) are time-course signals and \( \bar{x}_1 \) and \( \bar{x}_2 \) are the mean of \( x_1 \) and \( x_2 \), respectively. It takes values in the interval \([-1, 1]\) and measures the strength of the linear relationship between \( x_1 \) and \( x_2 \). It is worth noting that the dFC/dFNC pipeline's input is not the raw data, and it is usually preprocessed data. With \( N \) different nodes, this procedure constructs a symmetric \( N \times N \) connectivity matrix with \( N \times (N-1)/2 \) connectivity features in each window. Also, a tapered window has been suggested to alleviate the effect of sudden changes in using a rectangular window [4]. A tapered window is a convolution of two rectangular windows.

There are some drawbacks to the sliding window approach. The choice of window size is an implicit assumption about the dynamic behavior in that a short window captures more
rapid fluctuations, whereas a longer window does more smoothing than a shorter one. Also, having a reliable correlation value is challenging in a shorter window with a few samples. Therefore, a trade-off for having good specificities with a long window size to detect reliable dFC/dFNC changes and good sensitivity with a short window size is needed to avoid missing any desired dFC/dFNC variations. Even though there is no clear answer on choosing the best window size, the lower limit for avoiding undesired artifacts is setting the window size to the largest wavelength of the fMRI time courses. A window size between 30 to 60 seconds has been widely used in resting-state dFC/dFNC analysis.

A change-point detection approach has been proposed to eliminate the window size challenge. This approach finds the change in the FC/FNC state due to the change in signs of the image intensities at the adjacent time point [5]. Another study hypothesized that the sliding window method, which uses a fixed window size, cannot capture the connectivity pattern if there is a difference in the connectivity across frequencies. Addressing this problem, the mentioned study subdivided the brain time-course signals into a set of 78 frequency bins spanning the resting-state range, i.e., between 0.01 Hz to 0.1 Hz, and estimated a connectivity matrix for each. Graph analysis found a difference in within- and between-network connectivity across frequencies [6].

Although Pearson correlation was widely used in dFC/dFNC research, a recent paper explored other possible options, including Pearson, Spearman, and Kendall correlation, Pearson and Spearman partial correlation, Mutual Information (MI), Variation of Information (VI), Kullback–Leibler divergence, and Multiplication of Temporal Derivatives and Inverse Covariance for measuring the connectivity between different regions of the brain. This study showed that MI and VI yielded the most consistent results
by achieving high reliability concerning dFC/dFNC estimates for different window sizes [7].

1.1.2 Extracting dFC/dFNC states

After calculating the FC/FNC by applying the sliding window approach, we need to extract states to have an abstract representation of dFC/dFNC matrixes. Clustering analysis, particularly the k-means clustering method, is widely used for categorizing dFC/dFNC matrixes into a set of states [6]. In this method, we partition the connectivity matrix into $k$ clusters (called states here) in which each sample falls into the nearest cluster based on its distance from the cluster centroid. Although the k-means clustering method is relatively simple to implement, scalable to a large dataset, and guarantees convergence, it has drawbacks. For example, it needs a pre-defined number of clusters and is vulnerable to initial values that may yield different results. Also, the cluster centroid can be dragged by outliers.

Hierarchical clustering creates trees of clusters of samples, called a dendrogram, in which any two clusters are disjoint, or one includes the other. The cluster of all samples is the root of the tree. It does not require us to pre-specify the number of clusters to be generated as is needed for the k-means approach [8]. However, it also involves the definition of a specific threshold for cluster separation. Both k-means and Hierarchical clustering are not assumption-free and need a priori knowledge for categorizing the states of brain activity that may bias or affect the states' interpretation.

Recent studies replaced clustering with temporal independent component analysis (ICA) and principal component analysis (PCA) to eliminate the prior knowledge requirement. In the PCA-based approach, FCs/FNCs are linearly decomposed into a finite set of mutually
spatially orthogonal connectivity patterns [9]. In the ICA-based method, FCs/FNCs are decomposed into a finite set of connectivity patterns that are mutually temporally independent and have a linear contribution to the observed FCs/FNCs [10]. The main limitation of PCA is the linearity assumption. Then, PCA would fail if the variable in the dataset is not linearly correlated.

1.1.3 Temporal properties of dFC/dFNC

There are a few metrics by which we can model the temporal variation of dFC/dFNC. The simplest version of this model is FC's standard deviation across windows (time) [11]. Dwell time, the average amount of time that a subject spends in each state is a metric for modeling the temporal variation of dFC/dFNC in a state-based (or meta-state) approach [12]. The transition matrix contains the number of times a subject switches from one state to another is another metric that can be used to model the temporal changes of dFC/dFNC in a meta-state method [13]. Also, the largest distance between a meta-state occupied by a subject and the total distance traveled by a subject's brain during rs-fMRI scanning is another possible metric for modeling the temporal pattern of dFC/dFNC [14]. The Hub state is another metric proposed in the meta-state method, where the meta-state that a subject returns to four or more times is the hub state for that subject [15].

1.2 Clinical application of dFC/dFNC

1.2.1 Schizophrenia

Schizophrenia affects around 1% of the adult population in the world and around 2.4 million adults in the United States (Figure 1-3) [16]. Subjects with this disorder show abnormal dysconnectivity in functional and structural brain patterns [17]. The temporal feature of dFC/dFNC has been reported as a plausible biomarker in finding the fundamental
A previous whole-brain dynamic connectivity analysis showed that schizophrenia subjects spend less time in a highly-connected state [12], [22]. Another study from our group showed an abnormal pattern in the dFNC of the default mode network (DMN) by comparing state-based connectivity strength, dwell time, and between-state transition number of healthy control (HC) and schizophrenia (SZ) subjects [21]. This study identified SZ-associated patterns in the temporal dynamics of DMN in SZ subjects by showing that they spend more time in a state with sparsely connected nodes. This study also demonstrated a state-specific spatial disruption within DMN by showing that the central hubs of the posterior cingulate cortex and anterior medial prefrontal cortex are significantly impaired in SZ subjects. However, this study did not show how symptom severity is associated with this abnormal pattern. Also, another study showed the between-state transition number is significantly smaller for SZ subjects compared with that of HC subjects. Similarly, another study found that the whole-brain FNC pattern in markedly less dynamically active in SZ subject compared with that of HC subject. In more detail, it found that SZ subjects were found to exhibit diminished dynamic fluidity, visiting less meta-
states, shifting less often across them. This pattern is more pronounced in patients with high levels of hallucinatory behavior [15].

1.2.2 Major depressive disorder

Major depressive disorder (MDD) is a severe mood disorder characterized by feelings of sadness, anger, loss, diminished interests, and social withdrawal [23], [24]. MDD affects more than 16 million (Figure 1-3), or 6.7 percent, adults in the United States and 350 million, or 4.4 percent, adults worldwide each year [25]. Despite significant progress in treating MDD, 20% to 30% of patients are treatment-resistant [26]. To improve treatments, we need a better understanding of the underlying mechanisms of MDD. A study with a relatively large sample size of subjects (182 MDD patients and 218 HC subjects) analyzed the dynamics of the whole-brain FNC and found the MDD subjects spend more time in the state with lower FC in DMN, cognitive control network (CCN), and frontal network (FN). This study also found that HC subjects spend more time in the state with higher FN in the visual sensory network (VSN) [27]. Another study found that MDD patients showed a decreased dynamic between medial prefrontal cortical (mPFC) and parahippocampal gyrus within DMN, while they showed more dynamic in the connectivity between mPFC and insula [28]. Another study investigated the alteration of FC in DMN (between posterior cingulate cortex or PCC and mPFC) by looking at the standard deviation (connectivity variability) of dFNC thought was within a relatively small dataset [29]. This study found greater connectivity variability in MDD between mPFC and PCC. In contrast, another study found a lower connectivity variability in MDD in the connections between the DMN and the frontoparietal network [30]. Again, similar to SZ, the association between MDD symptom severity and dFNC features has not been explored yet.
1.2.3 Alzheimer's disease

Alzheimer's disease (AD) is the most common age-related dementia, affecting individuals 5.8 million adults over 65 years of age in the United States (Figure 1-3) [31]. It usually causes several deficits in memory, thinking, behavior, and social skills. AD usually progresses slowly in 3 stages, including mild cognitive impairment (early-stage), mild dementia (middle-stage), and severe dementia (late-stage) [32]. There is no way to treat AD, but some medications can decelerate its progress, particularly when it is detected in an early stage of AD [33]. Therefore, predicting AD progression and differentiating different stages of this disease is an essential step in early medical intervention in this mental disorder [34]–[39]. One study with 29 AD patients and 31 HC subjects found that AD patients spend more time than the HC subject in a state with sparse connectivity patterns in which the motor network is isolated from the rest of the brain. Also, the same study found an inability to switch out from a state with low inter-network connectivity into more highly connected network configurations in AD patients.

1.3 Limitations in dFNC analysis

Researchers have applied dynamic functional connectivity for a decade; there is still no comprehensive and unified toolbox to estimate dFNC features. The currently available dFNC pipeline is ill-suited for the large dFNC dataset. Developing a method for analyzing a massive dFNC dataset is needed. Most dFNC research was done on the whole-brain network [19], [40]. In a larger brain network, a group of brain networks such as visual, sensorimotor, and auditory networks, which are strongly correlated, may mask less-correlated networks and limit spatiotemporal resolution [41]. That potentially can delineate why these studies' main result was focused on these dominant networks and less reported
about the dominated network such as DMN. Although we can study dFNC of any brain network based on prior knowledge, a method that can mechanistically remove the irrelevant networks is needed [41]–[43]. Finally, while dFNC has been used in many neurological and psychiatric disorders, its clinical implications and benefits have not been well studied. The study of this thesis is trying to address all of the issues mentioned above.

1.4 Outline of the dissertation

This dissertation contains two parts. The first part includes four chapters and proposes new methods to analyze FNC information. In contrast, the second part includes six chapters discussing the clinical implications of analyzing dFNC information in neurological and neuropsychiatric disorders. The following paragraphs summarize the main contribution of each chapter.

Part 1: The first part talks about the methodological contribution of this dissertation and contains four chapters.

Chapter 2: iSparse k-means: A two-step clustering approach for big dynamic functional network connectivity data. The conventional dFNC pipeline is ill-suited for the big dFNC dataset. This chapter introduces a new dFNC pipeline that analyzes large dFNC information. We validate the proposed pipeline on four datasets from the same population of Human Connectome Project Young Adult or HCP-YA participants. We prove that our approach is 27 times faster than the conventional method in finding the clustering order.

Chapter 3: DyConx: A toolbox for extracting dynamic functional network connectivity features. Despite the extensive research of dFNC in finding neuroimaging biomarkers of different neuropsychiatric and neurological disorders, there is not yet an
open-source toolbox to estimate dFNC features. This chapter introduces a few new features that have not been explored before in dFNC. Additionally, this chapter introduces an open-source MATLAB toolbox called DyConx, that can help other researchers to extract dFNC features. Last but not least, in this chapter, we run the largest dFNC analysis using the UK Biobank dataset to validate the new dFNC features and DyConx.

Chapter 4: Recursive high influential connectivity removing for uncovering hidden dynamics. dFNC data extracted from rs-fMRI recordings have played a significant role in characterizing brain network interactions in various brain disorders and cognitive functions. dFNC analyses frequently use clustering methods to identify states of network activity. However, it is possible that these states are dominated by a few highly influential networks or nodes, which could obscure condition-related insights that might be gained from networks or nodes less influential to the clustering. This chapter presents an automatic dFNC pipeline based on feature learning to uncover network dynamics less influential to the initial clustering. We demonstrate the viability of our approach within the context of schizophrenia (SZ), applying our approach to a dataset consisting of 151 participants with SZ and 160 controls (HCs). We found that removing some connectivity pairs significantly affects the underlying states and magnifies the differences between participants with SZ and HCs in each state. Given our findings, we hope our approach will contribute to the characterization and improved diagnosis of various neurological conditions and functions.

Chapter 5: Visualizing functional network connectivity difference between healthy control and patient using the explainable machine learning method.

In recent decades, analyzing the FNC, obtained rs-fMRI techniques has revealed new information about the underlying neurophysiological mechanism of different neurological
and neuropsychiatric disorders. Many pieces of research focus on increasing classification accuracy between healthy and patient groups based on FNC information. However, developing a pipeline exploring the difference between healthy and patient groups FNC is less explored. This chapter develops a new pipeline based on an explainable machine learning approach called Shapley Additive explanation or SHAP that can find a subset of FNC features that contribute more than the other features in classification between healthy and patient groups. We validate the pipeline on a synthetic dataset. Next, we use the pipeline to find the underlying mechanism of schizophrenia and aging based on the FNC information.

Part 2: The second part talks about the clinical contribution of this dissertation and contains six chapters.

Chapter 6: Alzheimer’s disease projection from normal to mild dementia reflected in dynamic functional network connectivity. In this chapter, we explore the dFNC pattern in the AD progression. We use longitudinal rs-fMRI from 1385 scans (from 910 subjects) at different stages of AD (from normal to very mild AD or vmAD). We found that all brain states showed significant disruption during the progression from the normal brain to vmAD one. Specifically, we found that subcortical network, auditory network, visual network, sensorimotor network, and cerebellar network connectivity decrease in vmAD compared with those of a healthy brain. We also found reorganized patterns (i.e., both increases and decreases) in the cognitive control network and default mode network connectivity progression from normal to mild dementia.

Similarly, we found a reorganized pattern of between-network connectivity when the brain transits from normal to mild dementia. However, the connectivity between visual and
sensorimotor networks decreases in vmAD compared with a healthy brain. Finally, we found that a normal brain spends more time in a state with higher connectivity between visual and sensorimotor networks. Overall, this chapter provides new insights into the dFNC pattern changes in the progression of AD.

Chapter 7: The link between static and dynamic brain functional network connectivity and genetics risk of Alzheimer’s Disease. Apolipoprotein E (APOE) polymorphic alleles are genetic factors associated with AD risk. Although previous studies have explored the link between AD genetic risk and sFNC, no previous studies have evaluated the association between dFNC and AD genetic risk. This chapter examines the link between sFNC, dFNC, and AD genetic risk with a reproducible, data-driven approach. We use rs-fMRI, demographic, and APOE data from cognitively normal individuals ($N=894$) between 42 to 95 years of age (mean $= 70$ years). We divided individuals into low, moderate, and high-risk groups. Using Pearson correlation, we calculate sFNC across seven brain networks. We also calculate dFNC with a sliding window and Pearson correlation. Next, we put dFNCs into three distinct states with k-means clustering. Then, we calculate the amount of time each subject spent in each state, called occupancy rate or OCR. We compare both sFNC and OCR, estimated from dFNC, across individuals with different genetic risks and found that both sFNC and dFNC are related to AD genetic risk. We found that higher AD risk reduces within-visual sensory network (VSN) sFNC and that individuals with higher AD risk spend more time in a state with lower within-VSN dFNC. Additionally, we found that AD genetic risk affects whole-brain sFNC and dFNC in women but not in men. In conclusion, we presented novel insights into the links between sFNC, dFNC, and AD genetic risk.
Chapter 8: Aberrant dynamic functional network connectivity of default mode network in schizophrenia and links to symptom severity. Some studies have shown abnormal functional network connectivity in the default mode network (DMN) of individuals with schizophrenia, and more recent studies have demonstrated abnormal dFNC in individuals with schizophrenia. However, DMN dFNC and the link between abnormal DMN dFNC and symptom severity have not been well-characterized. This chapter analyzes rs fMRI data from subjects with schizophrenia (SZ) and healthy controls (HC) across two datasets independently. This is the first study to investigate DMN dFNC and its link to schizophrenia symptom severity. We identified reproducible neural states data-driven and demonstrated that the connectivity strength within those states differed between SZs and HCs. Additionally, we identified a relationship between SZ symptom severity and the dynamics of DMN functional connectivity. We validated our results across two datasets. These results support the potential of dFNC for use as a biomarker of schizophrenia and shed new light on the relationship between schizophrenia and DMN dynamics.

Chapter 9: Multiple overlapping dynamic patterns of the visual sensory network in schizophrenia. Although visual processing impairments have been explored in SZ, the underlying neurobiology of the visual processing impairments has not been widely studied. Also, while some research has hinted at differences in information transfer and flow in SZ, few investigations of functional connectivity dynamics within visual networks exist. This chapter analyzes rs-fMRI data of the visual sensory network (VSN) in 160 HC and 151 SZ. We estimated 9 independent components within the VSN. Then, we calculate the dFNC using the Pearson correlation. Next, using k-means clustering, we partition the dFNCs into
five distinct states, and then we OCR. We compare HC with SZ subjects and investigate the link between OCR and visual learning in SZ using OCR. Besides, we compare the VSN functional connectivity of SZ and HC in each state. We found that this network is indeed highly dynamic. Each state represents a unique connectivity pattern of fluctuations in VSN FNC, and all states showed significant disruption in SZ. Overall, HC showed stronger connectivity within the VSN in states. Subjects with SZ spent more time than HC in a state where the connectivity between the middle temporal gyrus and other regions of VNS is highly negative. Besides, OCR in a state with strong positive connectivity between the middle temporal gyrus and other regions correlated significantly with visual learning scores in SZ.

Chapter 10: Aberrant dynamic functional network connectivity of default mode network predicts symptom severity in major depressive disorder. MDD is a severe mental illness marked by a continuous sense of sadness and a loss of interest. The DMN is a group of brain areas that are more active during rest and deactivate when engaged in task-oriented activities. The DMN of MDD has been found to have aberrant sFNC in recent studies. This chapter extends previous findings by evaluating dFNC within the DMN subnodes in MDD. We analyzed rs-fMRI data of 262 patients with MDD and 277 HCs. We estimate dFNCs for seven subnodes of the DMN, including the anterior cingulate cortex (ACC), posterior cingulate cortex (PCC), and precuneus (PCu), using a sliding window approach, and then cluster the dFNCs into five brain states. Classification of MDD and HC subjects based on state-specific FC is performed using a logistic regression classifier. Transition probabilities between dFNC states were used to identify relationships between symptom severity and dFNC data in MDD patients. A disrupted connectivity
pattern was observed by comparing state-specific FNC between HC and MDD within the
DMN. In more detail, we found that the connectivity of ACC is stronger, and the
connectivity between PCu and PCC is weaker in individuals with MDD than in those HC
subjects. In addition, MDD showed a higher probability of transitioning from a state with
weaker ACC connectivity to a state with stronger ACC connectivity, and this abnormality
is associated with symptom severity. This is the first research to look at the dFNC of the
DMN in MDD with a large sample size. It provides novel evidence of abnormal time-
varying DMN configuration in MDD and links to symptom severity in MDD subjects.

Chapter 11: Dynamic functional network connectivity links with treatment response
of electroconvulsive therapy in major depressive disorders. Electroconvulsive therapy
(ECT) is one of the most effective treatments for major depressive disorder. Recently, there
has been increasing attention to evaluating the effect of ECT on rs-fMRI. This chapter aims
to compare rs-fMRI of patients with depression (DEP) with HCs, investigate whether pre-
ECT dFNC estimated from patients’ rs-fMRI is associated with an eventual ECT outcome,
and explore the effect of ECT on brain network states.

Rs-fMRI data are collected from 119 patients with depression or depressive disorder (DEP)
(76 females), and 61 healthy (HC) participants (34 females), with age, mean of 52.25 (N =
180) years old. The pre-ECT and post-ECT Hamilton depression rating scale (HDRS) were
25.59 ± 6.14 and 11.48 ± 9.07, respectively. Twenty-four independent components from
DMN and CCN are extracted using group-independent component analysis from pre-ECT
and post-ECT rs-fMRI. Then, the sliding window approach is used to estimate the pre-and
post-ECT dFNC of each subject. Next, k-means clustering is separately applied to pre-ECT
dFNC and post-ECT dFNC to assess the three distinct states of the whole group. We
calculate the OCR of each participant. Next, we compare OCR values between HC and DEP. We also calculate the partial correlation between pre-ECT OCRs and HDRS change while controlling for age, gender, and site. Finally, we evaluate the effectiveness of ECT by comparing pre-and post-ECT OCR of DEP and HC participants. Our finding suggests that dFNC features, estimated from CCN and DMN, show promise as a predictive biomarker of the ECT outcome of patients with depression. Also, this study identifies a possible underlying mechanism associated with the ECT effect on DEP patients.

**Chapter 12: Conclusions and future work.** This chapter discusses the main contribution of this Ph.D. dissertation and lists all publications from this thesis. Also, this chapter talks about the potential future direction based on the result of this Ph.D. dissertation.
PART 1:
METHODOLOGICAL CONTRIBUTIONS
CHAPTER 2. ISPARSE K-MEANS: A TWO-STEP CLUSTERING APPROACH FOR BIG DYNAMIC FUNCTIONAL NETWORK CONNECTIVITY DATA

2.1 Introduction

In recent decades, blood-oxygenation-level-dependent (BOLD) functional magnetic resonance imaging (fMRI) has provided unique information about brain changes associated with various brain disorders [3], [44], [45]. fMRI is a non-invasive imaging technique that identifies localized, time-varying alterations in brain metabolism, such as blood flow and deoxygenated hemoglobin levels [46]. These metabolic changes can be induced by a cognitive task (i.e., task-based fMRI) [47] or via unregulated brain fluctuations during rest (i.e., resting-state fMRI). Functional connectivity (FC) or its network analog functional network connectivity (FNC) studies the temporal dependence (typically assessed with correlation) between the BOLD fMRI signal from different brain regions [48]. The FNC approach uses temporal dependence to infer how various brain networks communicate and may play a significant role in understanding how large-scale neuronal communication in the human brain relates to human behavior [47], [49] and how neurodegenerative diseases alter this relationship [35], [50]–[53].

Most previous studies assume FNC is static over time and ignore (average out) brain dynamics [54]. Indeed, functional connectivity is highly dynamic, even during resting [55]. In recent years, a new line of research called dynamic functional network connectivity (dFNC) has moved beyond studying the strength of connectivity among brain regions and
studied the temporal properties of the FNC [4]. dFNC has shown promise as a biomarker for schizophrenia [56], [57], Alzheimer’s disease [55], major depressive disorder [58], and autism spectrum disorder [59]. It has been shown that dFNC improves the classification between disordered and healthy conditions [60], [61] and provides more information about the pathology of neurological and neuropsychiatric disorders than its static counterpart [62].

Figure 2-1 shows the analytic pipeline that is used for analyzing dFNC information [55]–[58], [60]. This pipeline contains four main steps. In the first step, we estimate the intrinsic components for the desired brain regions. Second, we calculate the dFNC using a sliding window. In the third step, we concatenate all dFNCs of all subjects and go through an optimization process to find the clustering order based on the elbow criterion. In the fourth

Figure 2-1: The conventional dFNC pipeline. In Step1, we estimate the independent components using group independent component analysis. In Step2, we estimate the dFNC using a sliding window. In Step3, we concatenate all dFNCs across all participants. Then, based on elbow criteria, we estimate the cluster order. In step4, we use a standard kmeans clustering approach and calculate the dFNC state for the group and state vector for everyone.
step, we estimate the final dFNC for the whole group and state vector for each individual and calculate the dFNC features for statistical analysis.

Even though any clustering approach can be used for clustering dFNC information, mainly k-means clustering has been used due to its simplicity in implementation ability to scale to a large dataset [63]. Additionally, it has been shown that k-means clustering is faster than the other methods such as spectral clustering, density-based spatial clustering of applications with noise or DBSCAN, and mean-shift clustering [64]. But it is still slow and needs substantial computational power when we work on a sizeable dFNC dataset. On the other hand, recently, the availability of extremely large neuroimaging datasets has made the computational burden of clustering dFNC measurements a significant practical challenge. For example, the UK Biobank dataset released neuroimaging data from more than 40,000 participants [65] and has targeted acquiring data from 100,000 individuals [66]. Also, it has been discussed that many neuroimaging analytic pipelines are not scalable for massive data sets, including possibly tens, if not hundreds of thousands of participants [67]. Therefore, developing a framework that can analyze a large dFNC dataset within a reasonable timeframe in a typical cluster computing environment is needed.

In this chapter, we introduce a new iterative clustering algorithm, iterative sparse k-means (iSparse k-means), that efficiently scales to millions of high-dimensional observations, making it a valuable addition to the pipeline for large scale dFNC analyses. We evaluated the reproducibility of the results with both standard and proposed dFNC pipelines across four rs-fMRI sessions of HCP young adults. Additionally, we compared the time needed to find the optimal cluster number with iSparse k-means versus standard k-means, and showed that our approach is faster than the standard method in finding the cluster order.
2.2 Materials and methods

Our analytic pipeline includes rs-fMRI preprocessing, extracting independent components, calculating dFNC, and estimating the cluster order and dFNC states using the proposed clustering method. The following subsection describes each step in more detail.

2.2.1 Preprocessing and independent components extraction

We used the statistical parametric mapping (SPM12, https://www.fil.ion.ucl.ac.uk/spm/) running in MATLAB2019 to preprocess the fMRI data. The first five dummy scans were removed before preprocessing. Rigid body motion correction was used to account for participant's head movement. Then, we used spatial normalization by echo-planar imaging (EPI) template in the standard Montreal Neurological Institute (MNI) space. Finally, a

Figure 2-2: Extracted independent components. 53 independent components estimated by NeuroMark pipeline. We put them in seven domains, including subcortical network (SCN), auditory network (AND), sensorimotor network (SMN), visual sensory network (VSN), cognitive control network (CCN), default mode network (DMN), and cerebellar network (CBN).
## Table 2-1 Component labels

<table>
<thead>
<tr>
<th>Component name</th>
<th>Peak coordinate (mm)</th>
</tr>
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<tbody>
<tr>
<td><strong>Caudate (69)</strong></td>
<td>6.5 10.5 5.5</td>
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<tr>
<td><strong>Subthalamus/hypothalamus (53)</strong></td>
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<td><strong>Putamen (98)</strong></td>
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<td><strong>Caudate (99)</strong></td>
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<tr>
<td><strong>Thalamus (45)</strong></td>
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<tr>
<td><strong>Superior temporal gyrus ([STG], 21)</strong></td>
<td>62.5 -22.5 7.5</td>
</tr>
<tr>
<td><strong>Middle temporal gyrus ([MTG], 56)</strong></td>
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</tr>
<tr>
<td><strong>Postcentral gyrus ([PoCG], 3)</strong></td>
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</tr>
<tr>
<td><strong>Left postcentral gyrus ([L PoCG], 9)</strong></td>
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</tr>
<tr>
<td><strong>Paracentral lobule ([ParaCL], 2)</strong></td>
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</tr>
<tr>
<td><strong>Right postcentral gyrus ([R PoCG], 11)</strong></td>
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</tr>
<tr>
<td><strong>Superior parietal lobule ([SPL], 27)</strong></td>
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</tr>
<tr>
<td><strong>Paracentral lobule ([ParaCL], 54)</strong></td>
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<tr>
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<tr>
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<td>Middle cingulate cortex ([MCC], 37)</td>
</tr>
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<tr>
<td>41</td>
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<tr>
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<tr>
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Gaussian kernel was used to smooth the fMRI images using a full width at half maximum (FWHM) of 6mm.

Next, we adapted the Neuromark pipeline to extract intrinsic connectivity networks (ICNs) for each subject \[68\]. Using this pipeline, we estimated 53 ICNs for each subject and categorized them into seven network domains, including subcortical network (SCN), auditory network (ADN), sensorimotor network (SMN), visual network (VSN), cognitive control network (CCN), the default-mode network (DMN), and cerebellar network (CBN) as shown in Figure 2-2. The details of the extracted ICNs are provided in Table 2-1.

2.2.2 Dynamic functional network connectivity estimation

We used a tapered sliding window and estimated the functional connectivity within each window using the Pearson correlation as shown in Equation (2.1).

\[
R = \frac{\sum_{n=1}^{N}(x_1 - \bar{x}_1)(x_2 - \bar{x}_2)}{\sqrt{\sum_{n=1}^{N}(x_1 - \bar{x}_1)^2} \sqrt{\sum_{n=1}^{N}(x_2 - \bar{x}_2)^2}} \tag{2.1}
\]

where \(x_1\) and \(x_2\) are time-course signals and \(\bar{x}_1\) and \(\bar{x}_2\) are the mean of \(x_1\) and \(x_2\), respectively. It takes values in the interval \([-1, 1]\) and measures the strength of the linear relationship between \(x_1\) and \(x_2\).

With 53 ICN, the size of each dFNC is \(53 \times 53\), which equals 1378 distinct connectivity features. Next, we concatenated dFNC estimates of each window for each subject to form a matrix, called dFNC tensor hereafter, with the size of \(T \times F\), where \(T\) denotes the number of windows and \(F\) donates the number of connectivity features (Figure 2-3).

2.2.3 iSparse k-means clustering
Figure 2-3: The overview of iSparse k-means clustering approach for dFNC state estimation. In Step 1, we select a subsample of dFNC tensor and then used kmeans clustering with k values from 2 to L and put them into \( \frac{L(L-1)}{2} - 1 \). With r iteration, we would have \( r\left(\frac{K(K-1)}{2} - 1\right) \) clusters centroids in total. In Step 2, we concatenated all cluster centroids, and we used elbow criteria to find the best k values, called \( K_{opt} \) hereafter. In Step3, using another kmeans clustering approach, we estimated the final dFNC states. In Step 4, we used these final states and found the state vector for each subject.

Figure 2-3 shows the proposed iSparse k-means clustering method for estimating dFNC states. This method includes a few steps. **Step1:** We sub-sample subjects dFNC tensors (m subjects from n subjects per iteration). Then, we run a standard k-means clustering on the subsampled data with different values of \( k = 2, 3, \ldots, L \). The k-means algorithm divides \( m \times T \) samples \( X \) of each iteration into \( k \) disjoint clusters \( C_1, C_2, \ldots, C_k \). The cluster centroids \( \mu_l \) of \( C_l \) minimize the within-cluster sum-of-squares criterion as shown in Equation (2.2).
\[
\min_{\{\mu_1, \ldots, \mu_k\}} \left( \sum_{j=1}^{k} \sum_{t=1}^{mT} (\|x_{it} - \mu_j\|^2) \right)
\]

We exhaust all subjects by repeating this process \(r\) times over disjoint sets of \(m\) subjects, where \(r\) is equal to \(\frac{n}{m}\). In each iteration, we save all cluster centroids for all values of \(k \in [2, L]\). Therefore, we would have \(\frac{L(L+1)}{2} - 1\) representative cluster centroids in each iteration. By repeating this process \(r\) times, we would have \(r\left(\frac{L(L+1)}{2} - 1\right)\) cluster centroids, a reduction of the data from the whole dFNC. **Step 2:** We concatenate all centroids estimated from all \(r\) iterations. Next, we use the elbow criteria to find the optimum number of clusters using all \(r\left(\frac{L(L+1)}{2} - 1\right)\) observations. **Step 3:** After finding the optimum number of clusters, called \(K_{opt}\) hereafter, we use another standard k-means clustering to put all \(r\left(\frac{L(L+1)}{2} - 1\right)\) states into \(K_{opt}\) cluster, called final states. **Step 4:** Using the final \(K_{opt}\) states, we assign the dFNC of each subject to one of the estimated states and extract the state vector of each participant.

### 2.2.4 dFNC temporal features estimation

We estimated the occupancy rate (OCR) and the number of transitions between states as the representative dFNC temporal features from the state vector. The OCR represents the proportional amount of time each individual spends in a given state for all HCP datasets through both standard and isparse k-means methods.

### 2.2.5 Clustering quality assessment

To assess the clustering quality for each dFNC data, we calculated the distance between the dFNC data and its associated cluster centroid. Then we calculated the distance
between each dFNC sample with the other cluster centroids and then summed them up. Then, we calculated the ratio of the latter to the former one for each dFNC instance, called the distance ratio here. Finally, we averaged all distance ratios out for each participant.

\[ R_p = \frac{1}{T} \sum_{i=1}^{T} \frac{d_{i,sc}}{d_{i,c}} \]

\( d_{i,c} \) is the distance between each sample to the cluster centroid of the state the sample belongs. Also, \( d_{i,sc} \) is the distance between each sample to other cluster centroids, \( R_p \) is the averaged distance ratio for each participant. It is worth mentioning that a higher ratio means better quality in clustering.

2.2.6 Dataset

To test the proposed method, we used the rs-fMRI and demographic information collected from the 833 young healthy adults (average age: 28.65; range: 22-37 years; female/male: 443/390) from the Human Connectome Project (HCP) [69]. This dataset is available on the HCP website (https://www.humanconnectome.org). The institutional review board from both Washington University and the University of Minnesota approved the study. The rs-fMRI data were collected on a Siemens Skyra 3T with a 32-channel RF receiver head coil. High resolution T2*-weighted functional images were acquired using a gradient-echo EPI sequence with TE = 33.1 ms, TR = 0.72 s, flip angle = 52°, slice thickness = 2 mm, 72 slices, and 2 mm isotropic voxel, the field of view: 208×180 mm (RO×PE), and duration: 14:33 (min: sec). For each participant, four separated rs-fMRI sessions (two sessions per day) were acquired that are called HCP1 (session1, day1), HCP2 (session2, day1), HCP3 (session 1, day2), and HCP4 (session2, day12), hereafter. We used all four
sessions to evaluate the reproducibility of the result using the proposed dFNC states estimation method. The dFNC size of HCP1, HCP2, HCP3, and HCP4 is 848827×1378 (8542 MB), 732207×1378 (7403 MB), 747201×1378 (7555 MB), and 769692×1378 (7742 MB), respectively.

2.3 Results

2.3.1 Standard k-means and iSparse k-means clustering produce similar brain states.

The first question we were interested in answering is whether both standard k-means and iSparse k-means would generate similar dFNC states or not. To test this, we clustered the dFNC data with different L values in iSparse k-means (as shown in Figure 2-3). In the
iSparse k-means, we used 3% of the entire dataset in each iteration. Using elbow criteria, we found that the optimal number of clusters is 2 through both standard and proposed k-means clustering approaches. Then, to evaluate the similarity of dFNC states estimated by iSparse k-means (with different $L$) with the states estimated by conventional k-means, we used the correlation across the matched states as a similarity metric. The similarity between matched states with varying values of $L$ is shown in Figure 2-4A for all four HCP datasets. We found that the similarity between the matched states generated by both approaches is more than 99%, with any value $L$ of more than five, and the results were reproduced across four HCP datasets. The estimated states with conventional k-means and iSparse k-means ($L=6$) are shown in Figure 2-4B for all HCP datasets.

**Figure 2-5**: The clustering evaluation time with conventional and iSparse kmeans method. Reducing the percentage of the data used in each iteration of the first step reduces the evaluation time. The iSparse kmeans method is 27 times faster than the conventional method. The estimated states and their similarity with states estimated from whole data are shown for each percentage of data.
### 2.3.2 iSparse k-means finds the optimum cluster number faster than the conventional k-means

After finding the minimum reliable value of L, we assessed the speed of our method in finding the optimum number of clusters and compared it with the conventional method when it uses the whole dataset. We evaluated the speed of our process with different percentages of data. The results are shown in Figure 2-5 for HCP1.

We found that iSparse k-means is faster when we use a lower percentage of data in each iteration, while the similarity between the matched states estimated with both standard k-means and iSparse is still more than 98%. Additionally, our proposed method is 27 times faster in funding the cluster order than the traditional method when we use only 0.12% of data (one subject) in each iteration.

### 2.3.3 iSparse k-means and conventional k-means generate similar dFNC features.

**Figure 2-6**: Both standard kmeans and iSparse kmeans generated similar dFNC features replicated across four datasets. A) Estimated number of transitions from both standard kmeans and iSparse kmeans for all HCP datasets. The similarity between the estimated number of transitions from both methods is more than 0.989. B) Estimated occupancy rate (OCR) from both standard kmeans and iSparse kmeans for all HCP datasets. The similarity between the OCR from both methods is more than 0.989.
The next question is whether both clustering approaches generate similar dFNC features or not. We estimated occupancy rate or OCR, the proportional amount of time each participant spends in a specific state, and the number of between-state transition numbers for each participant in both standard and iSparse k-means. Both features are estimated from the state vector, which shows the state of the brain at a given time (Figure 2-3 step4). Then, to assess the similarity between the two methods in estimated dFNC features, we calculated the correlation between the result of the two methods. The results are shown in Figure 2-6A and Figure 2-6B for OCR and the number of transitions, respectively, for all four HCP datasets. As Figure 2-6A shows, the correlation between the estimated OCR by k-means and iSparse k-means is more than 0.98 (p<e^{-10}). The result was replicated for all four HCP datasets. Additionally, the number of between-state transitions is significantly similar for both methods, and the result was repeated in all HCP datasets. This piece of evidence shows that our new clustering method produced similar dFNC features as well as the standard k-means while our method is faster in finding the clustering order and does not require prohibitive levels of computational power.

2.3.4 iSparse k-means has better cluster quality than the standard k-means
**Figure 2-7** shows the distance ratio of both standard and iSparse k-means with different $k$ values in all four HCP sessions. We used a two-sample t-test to compare the distance ratio of standard k-means vs. iSparse one. We found iSparse k-means would have better cluster quality than the standard one in all comparisons by having a higher distance ratio.

### 2.4 Discussion

In this study, we developed an analytic pipeline to analyze large data dFNC information even without having a sophisticated computational resource. There are a few benefits of using this novel framework. 1) in the standard k-means approach, we need to load the entire dataset, which can be computationally demanding and slow when using a large dFNC dataset. Our proposed method does not require loading the entire dataset. This dramatically reduces the required computational resources, 2) we showed our method is 27 times faster than the standard method in finding the cluster order, 3) we validated the reproducibility of the result across four sessions of rs-fMRI data within a population group; and 4) we demonstrated that our approach generates improved clustering quality compared to the standard approach.

Unlike standard k-means, in which we need to load the entire dataset, our approach loads a portion of the data in each iteration. Therefore, we reduce both the required memory as well as the computational time. In this respect, our proposed algorithm is similar to mini-batch k-means, which partially loads the data and does not need expensive computational resources. But as [70] shows, the cluster quality for mini-batch k-means is reduced compared to standard k-means clustering, especially when the number of clusters increases. Unlike the mini-batch k-means approach, iSparse k-means reduces the entire clustering process time (Figure 2-5) and increases the clustering quality (Figure 2-7). recent
approaches for k-means clustering of big data have focused on identifying the most informative features for the dataset and then running a k-means on the reduced set. For example, a recent study reduced the dimension of the data set from $p$ to $m$ ($p>m$) by applying a principal component analysis on the entire dataset followed by k-means clustering on the projected dataset [71]. This method still needs the whole dataset to be loaded, which requires massive computational power. Additionally, since the k-means is applied to the project space, we do not have an estimation of the cluster centroid in the original space. However, we can transfer the cluster centroid to the original space, but this estimate is inaccurate and yield lower cluster quality than the standard k-means approach.

Our dFNC pipeline is based on the Neuromark pipeline, a fully automated independent component analysis (ICA) framework that uses spatially constrained ICA to estimate components that are flexible to each subject's data and comparable across individuals [68]. Using the Neuromark pipeline, we calculated the replicated independent components for four hcp sessions. Additionally, we showed that 1) both standard and iSparse k-means generated similar dFNC states in each session of hcp data, 2) the brain states were replicated across all four sessions using both standard and the proposed k-means clustering approach. The reproducibility of the result across four sessions assessed the robustness of the proposed dFNC pipeline.

2.4.1 Limitations and future work

There are a few limitations to this study. First, our clustering method is not limited to k-means clustering. We can adapt other fast clustering approaches to this pipeline and further improve the computational speed. Second, we did not compare our method's computational
speed and clustering quality with other existing fast clustering approaches. However, unlike these fast methods, we showed that our approach generated a better-quality cluster than the standard k-means clustering method. A future study is needed to compare the results across multiple clustering approaches. Third, we did not propose an algorithmic approach to set the maximum $L$ value (Figure 2-3). Finding the optimum $L$ values is done empirically by running the method multiple times to evaluate replicability at different values of $L$.

2.4.2 Conclusion

Previous dFNC analytics pipelines use standard k-means clustering, which is ill-suited for big dFNC data. Here, we developed a new method called iSparse k-means clustering that reduced the evaluation time for finding the cluster order while we only loaded a portion of the dataset through several iterations. Therefore, in our new method, we do not need access to a strong computational power, as we need in the standard way for an extensive dataset. We validated that our method produces similar brain states and dFNC features as the standard method. Additionally, we evaluated the reproducibility of results across four HCP young adult datasets, which showed the high robustness of the proposed method.
CHAPTER 3. DYCONX: A TOOLBOX FOR EXTRACTING DYNAMIC FUNCTIONAL NETWORK CONNECTIVITY FEATURES

3.1 Introduction

Despite the extensive research in dynamic functional network connectivity (dFNC) usage in finding neuroimaging biomarkers of different neuropsychiatric and neurological disorders, there is not yet an open-source toolbox to estimate dFNC features. Therefore, a toolbox to calculate dFNC features is necessary.

This chapter aims at two goals. 1) It introduces new dFNC features, 2) develops an open-source toolbox in MATLAB to help other researchers to estimate dFNC features. We validated the new features and toolbox using the UK Biobank dataset.

3.2 Materials and methods

3.2.1 dFNC features estimation in DyConx

Here we introduce different functions in DyConx while introducing the new dFNC features.

3.2.1.1 The occupancy rate (OCR)

The occupancy rate or OCR is the proportional time each participant spends in any given state. It is the ratio of the number of windows in state \( i \) (i.e., \( T_i \)) to the total amount of windows (i.e., \( T \)).

\[
OCR_{ij} = \frac{T_{ij}}{T} \quad (3.1)
\]

in which \( T_{ij} \) is the number of windows of subject \( i \) in state \( j \), \( T \) is the total number of windows and \( OCR_{ij} \) is the occupancy rate in state \( j \) for each participant \( i \). In DyConx, the
MATLAB function called "ocr_estimation.m" will calculate the OCR for each subject. The inputs of this function include "state_vec", "num_of_state", "T", and "num_of_sub". "state_vec" is a column vector, "T" is a column vector that contains the total number of the window for each subject. The output of this function includes "ocr" a matrix with the size of num_of_sub×num_of_state, containing each subject's estimated OCR.

3.2.1.2 Maximum OCR in each state

This new feature estimates the maximum proportional amount of time that each subject spends in each state. The function called “max_ocr_in_state_estimation.m” estimates the maximum OCR of each subject at any state.

3.2.1.3 Maximum OCR in all states

This feature is the maximum amount of time each subject spends among all states. In DyConx, “max_ocr_estimation.m” calculates these new features.

3.2.1.4 Number of transitions to a specific state

Another timing-based feature that we can estimate from the state vector is the number to transition to a specific state. The function called “num_trans_to_a_state_estimation.m” will estimate this feature. The input of this function is the same as the “ocr_estimation.m”. The output is “num_trans_to_a_state”, a matrix with the size of num_of_sub×num_of_state. This matrix contains the estimated number of transitions to each state for each subject.

3.2.1.5 Total number of between-state transition
This feature estimates the total number of transitions among all states. The function called “num_trans_estimation.m” estimates this feature. The input of this function is the same as "ocr_estimation.m" and the output is a column vector with the size of num_of_sub×1.

3.2.1.6 The total dFNC traveled distance

The dFNC traveled distance during the entire rs-fMRI recording can be calculated using the equation below:

\[ D_i = \sum_{t=1}^{T-1} |X_{t+1} - X_t| \quad 3.2 \]

where \( X_t \) and \( X_{t+1} \) represent the FNC vector at the time \( t \) and \( t+1 \). The size of \( X \) is \( \frac{N(N-1)}{2} \), where \( N \) is the number of independent components. This feature can be estimated from the function called “traveled_dist_estimation_wo_state”. The inputs include “data”, “T”, “num_of_sub”, and “dist_type”. Here, data is the dFNC data, and “dist_type” is the type of distance that calculates the distance between any subsequent dFNC window. The distance type includes Euclidian, correlation, cosine, and Manhattan distance.

The Euclidian distance between \( X = [X_1, X_2, ..., X_N] \) and \( Y = [Y_1, Y_2, ..., Y_N] \) can be calculated by:

\[ d(X,Y) = \sqrt{\sum_{i=1}^{N} (X_i - Y_i)^2} \quad 3.3 \]

The correlation distance between \( X \) and \( Y \) is:

\[ d(X,Y) = 1 - \frac{\sum_{i=1}^{N} (X_i - \bar{X})(Y_i - \bar{Y})}{\sqrt{\sum_{i=1}^{N} (X_i - \bar{X})^2 \sum_{i=1}^{N} (Y_i - \bar{Y})^2}} \quad 3.4 \]

The cosine distance is
\[ d(X, Y) = \frac{\sum_{i=1}^{N} X_i Y_i}{\sqrt{\sum_{i=1}^{N} X_i^2 \sum_{i=1}^{N} Y_i^2}} \quad 3.5 \]

The Manhattan distance between \( X \) and \( Y \) is

\[ (X, Y) = \sum_{i=1}^{N} |X_i - Y_i| \quad 3.6 \]

The output of this function is “traveled_dist”, which is a column vector containing the traveled distance by each participant. It is worth noting that this feature is estimated from the state vector.

3.2.1.7 The total dFNC traveled distance in each state

A new dFNC feature we proposed here is the traveled distance by each participant in each state (Figure 3-1A). This new feature estimates the distance between consecutive dFNC data in each state based on the equation below.

\[ D_{ij} = \sum_{t=1}^{T-1} |X_{(t+1)j} - X_{tj}| \quad 3.7 \]

where \( D_{ij} \) is the estimated traveled distance of subject \( i \) in state \( j \).

3.2.1.8 Speed in each state

Another new feature we can estimate from both dFNC data and state vector is the subject speed in each state. The total traveled distance calculates in each state by the amount of time each subject spends in that state.

\[ SP_{ij} = \frac{D_{ij}}{T_{ij}} \quad 3.8 \]

where \( D_{ij} \) is the traveled distance by subject \( i \) in state \( j \), \( T_{ij} \) is the amount of time subject \( i \) spends in state \( j \), and \( SP_{ij} \) is the speed of subject \( i \) in state \( j \).
Figure 3-1: The new proposed dFNC features. A) The dFNC traveled distance for each participant in each state, B) The total movement traveled by each subject in each state, C) The distance between each dFNC and the state center, D) The size of the state for each subject calculated by the distance between any dFNC of each subject in each state. In this graph, each dFNC data are represented by each circle.
3.2.1.9 **The total dFNC movement**

We estimate the total dFNC movement by calculating the distance between the first dFNC and last dFNC data, as shown in Figure 3-1B. The total dFNC movement is the distance between each subject's first and last dFNC.

$$ M_i = |X_1 - X_T| $$

where $X_1$ is the first dFNC and $X_T$ is the last dFNC of each subject. In DyConx, “total_movement_estimation_wo_state” will measure the total movement of each subject. The output of this function is a column vector containing

3.2.1.10 **The total dFNC movement in each state**

The total dFNC movement in each state is the distance between each subject's first and last dFNC in any state, as shown in Figure 3-1B. “total_movement_in_state_estimation” calculates the total movement of each subject in each state. The output of this function is a matrix containing the total dFNC movement of each subject at each state.

3.2.1.11 **Distance to the cluster centroid**

To estimate the state's size for each subject, we first calculate the distance between and dFNC belonging to a participant to the cluster centroid in each state (Figure 3-1C), and then we add them up. The function called “state_dist_to_center_estimation” estimates this feature.

3.2.1.12 **Distance to the cluster centroid**
Another way to estimate the state size is by calculating the distance between any pair of dFNCs belonging to a subject in each state (Figure 3-1D), and then we add all distances.
up. In DyConx, the function called “state_size_estimation” estimates the state size. Table 3-1 shows the input and output of each function in DyConx.

3.2.2 Dataset

UK Biobank dataset was used in this study to evaluate the proposed dFNC features and the toolbox. We used the resting-state fMRI (duration: 5min) data of 37,784 (20,157 females) adults’ brains, demographic information (age:64.06± 7.51), and cognitive scores from the UK Biobank in which the cognitive scores include fluid intelligence (FI), reaction time (RT), and pairs matching (Pairs).

In “FI” test, which assesses verbal and numerical reasoning, the participants were required to answer 13 multiple-choice questions assessing verbal (e.g., “Bud is to flower as the child is to?” Possible answers: Grow/Develop/Improve/Adult/Old) and numerical (e.g., “150…137…125…114…104… What comes next?” Possible answers: 96/95/94/93/92) abilities. Each question was shown at the top of the computer screen, with 3–5 alternative responses beneath it (described here: https://biobank.ctsu.ox.ac.uk/crystal/field.cgi?id=20016). Participants were asked to choose which responses they believed were accurate, "Do not know" or "Prefer not to answer." The score is determined by the number of questions properly answered in two minutes.

For “RT” test, participants completed a timed test of symbol matching, like the common card game ‘Snap’ (described here http://biobank.ctsu.ox.ac.uk/crystal/field.cgi?id=20023). The mean response time in milliseconds across trials with matching pairings was used to calculate the score for this test.
In “Pairs” test, the participants were instructed to remember the placements of six card pairs and then match them from memory with the fewest possible mistakes (described here https://biobank.ctsu.ox.ac.uk/crystal/field.cgi?id=400). The pairs-matching test results are based on the number of mistakes made by each participant; hence, higher scores indicate lower cognitive performance. There were two versions of the pair matching task: 3-pair and 6-pair. Because there was more room for score variance in the 6-pair version, we concentrated our investigation on it. The detail of cognitive tests in the UK Biobank is comprehensively described in [72].

Using Neuromark, we adapted group independent component analysis to extract 53 data-driven components for the whole brain (see Figure 2-2 and Table 2-1). Next, we used the sliding window and Pearson correlation to estimate the dFNC among 53 components. We used iSparse k-means pipeline to estimate the clustering order. We found the optimum number of clusters is three. Then, we put all dFNCs into three states and estimated the state vector of each participant. Then, we estimated 30 dFNC features based on three estimated states and state vectors. Finally, we trained a two-fold cross-validation support vector regression (SVR) to predict the cognitive scores.

3.3 Results

3.3.1 Dynamic functional connectivity states

Figure 3-2 shows the reoccurring connectivity states identified by the iSparse k-means clustering method. In all states, we observed strong positive connectivity within ADN, SMN, VSN, and CBN. State 1 showed the strongest connectivity within SMN and within VSN among all states. In addition, this state had the highest connectivity between SMN and VSN. Also, this state was separated from other states by showing the lowest negative
connectivity between SMN and VSN with the rest of the brain. State 2 showed the lowest connectivity between SMN and VSN. Also, state 3 shows higher within sensory network connectivity than state 2 and lower one than state 1. Results showed that subjects spent an average of 13.66 %, 57.59 %, and 28.75 % in state 1, state 2, and state 3, respectively.

3.3.2 dFNC features predict cognitive scores in UK Biobank

We trained SVR with linear kernel function to predict UK Biobank cognitive score with estimated dFNC features. After predicting cognitive scores with the trained model, we calculated the correlation between the measured score with the predicted one. Figure 3-3A shows the correlation between the measured FI score versus the predicted one. As this figure shows, we found that dFNC features successfully predict the FI score ($R=0.043, p=6.6e^{-17}$). Figure 3-3B shows the correlation between measured RT mean time with predicted RT mean time, which shows dFNC features predict the RT mean time

![Figure 3-2: Dynamic functional connectivity states result.](image)

The three identified dFNC states using the iSparse k-means clustering method. We found strong connectivity within-ADN, within-SMN, and within-VSN in all states. We found strong connectivity between SMN and VSN in state 1 and state 3. Also, these two states showed negative connectivity between sensory networks, including ADN, SMN, and VSN, with the rest of the brain. We found all subjects spend 13.66 %, 57.59 %, and 28.75 % in state 1, state 2, and state 3, respectively. The color bar shows the strength of the connectivity. SCN: Subcortical network, ADN: auditory network, SMN: sensorimotor network, VSN: visual network, CCN: cognitive control network, DMN: default-mode network, and CBN: cerebellar network.
Finally, we found dFNC feature predicts the completion time in the pair matching test, as shown in Figure 3-3C ($R=0.049$, $p=1.3\times10^{-20}$).

### 3.3.3 Partial correlation analysis results

Next, we calculated the partial correlation between 30 dFNC features and the cognitive score while controlling for age, gender, and site. The heatmap that represents the links between dFNC features and cognitive scores is shown in Figure 3-4. In this figure, the colormap represents the link between the dFNC features and cognitive scores. Any correlation that survived false discovery rate (FDR) correction is shown with a box [73]. Also, the new dFNC features introduced in this chapter are shown in red. This figure shows that both old and new features significantly link with the FI score. However, more new features than the old ones show significant results. The following lines list the significant correlation between dFNC features and FI score.
1- OCR of state 2 shows a positive link with FI score. Those participants who spend more time in state 2 show higher FI scores. The main characteristic of this state was having relatively higher connectivity between sensory networks and the rest of the brain when we compared it with other states.

2- OCR of state 3 shows a negative link with FI score. That means those participants who spend more time in this state show lower FI scores.

3- Max ocr in state of state 2 has a positive link with FI score. Max ocr in state represents the maximum amount of time each subject continuously spends in each state. Our results show that those participants who stay in state 2 for more time than others show better performance in the FI test.

4- Traveled dist of state 2 has a positive link with FI score. This means those participants who traveled more or have more FNC change in this state will perform better in the FI test.

5- Traveled dist of state 3 shows a negative link with FI score, which means that participants with less FNC change in this state have less FI score.

6- Speed in state and movement speed in state of state 3 link negatively with FI score. This means that participants who change faster than those in state 3 have lower FI scores.

7- Dist to center of state 3 shows a negative link with FI score. That means that those close to the cluster centroid of state 3 have a better FI score.

8- Dist to center of dFNC of those participants is bigger than others have less FI score.
Figure 3-4: The correlation between dFNC features and UK Biobank cognitive scores. We estimated the partial correlation between 30 dFNC features and cognitive scores by controlling age and sex. The colormap represents the correlation between each dFNC feature and cognitive score. Those links that survived after FDR are shown in a square box. The new dFNC features are shown in red.

9- Total_movement and traveled_dist have a negative link with FI score. Those participants who get more change in dFNC in the entire scan time have lower FI scores.

In general, it seems state 2 shows more role than other states in the FI test. Additionally, only maxocr_in_state of state 1 shows a significant link with Pair completion time for Pair. This means that those participants who spend more time continuously in this state need more time to complete the pair matching test. Also, we did not observe any significant link between all dFNC features and RT mean time.

3.4 Discussion

Although significant research has been done on dFNC in recent years, a comprehensive toolbox that extracts dFNC features is still needed. Also, there are many ways to look at the dFNC proprieties which are less explored. This chapter contributes to the field in three
aspects: 1) it introduces a few new dFNC features that have not been explored before, 2) it introduces an open-source toolbox called DyConx that can extract dFNC features, and 3) it validated the new features and toolbox in the UK Biobank dataset containing 37,784 participants. To the best of our knowledge, this is the largest dFNC study that has ever been done.

We trained an SVR model only on 30 dFNC features extracted from three states and showed that the dFNC features could successfully predict the behavioral outcome in UK Biobank. However, we ran a partial correlation analysis by controlling age, sex, and site. We found that dFNC features only show a significant link with FI score, and only one dFNC feature significantly correlates with RT score. In other words, while a machine learning model trained on dFNC features predicts RT mean time and Pair matching completion time, the conventional statistical learning does not show a significant between dFNC features with RT mean time and Pair matching completion time. Two reasons might explain this discrepancy. First, in statistical learning, we added age as a covariate. It seems age has a significant contribution to the result. When we remove that from the partial correlation analysis, we can easily see a few significant links between dFNC feature with RT and Pairs scores. Moreover, statistical learning may become less precise as the number of input variables increases. On the other hand, machine learning methods focus on identifying generalizable patterns in dimensional data that can be used to make out-of-sample predictions [74].

3.4.1 Limitations and future work

There are a few limitations associated with the work discussed in this chapter. Our toolbox is function-based in MATLAB. Using this toolbox might be challenging for researchers
with limited experience with MATLAB. Future work is needed on a graphic user interface or GUI-based toolbox. Also, a GUI-based DyConx can be adapted better to GIFT. Also, making the toolbox in other programming languages such as Python would be beneficial. Also, we intend to add the graph-based estimation functions to the toolbox in the future.

3.4.2 Conclusion

Here, we introduced an open-source toolbox while introducing new dFNC features. We validated the toolbox and new features in the largest dFNC study using the UK Biobank neuroimaging dataset. We showed the new dFNC features estimated by the new toolbox successfully estimated the UKBiobank cognition.
CHAPTER 4. RECURSIVE HIGH INFLUENTIAL CONNECTIVITY REMOVING FOR UNCOVERING HIDDEN DYNAMICS

4.1 Introduction

In recent years, dynamic functional network connectivity (dFNC) extracted from resting-state functional magnetic resonance imaging (rs-fMRI) data has provided novel insights into many neurological conditions like schizophrenia (SZ) [55], [75], Alzheimer’s disease [55], [76], and major depressive disorder [77]. While multiple dFNC analysis methods have been developed, many studies have used clustering to identify neurological states [78]. After clustering, studies typically characterize the states and examine the relationship between the dynamics of the states and scores associated with various neurological disorders or cognitive functions. These studies have provided many novel insights. However, when multidimensional data is used, it is natural that some dimensions would have a greater influence upon the resulting clusters than others or that some networks would have more influence than others (Figure 4-1A).

Additionally, the influence of those dimensions or networks may obscure relevant activity in other less influential networks or dimensions. We have found evidence of this in our previous research. For example, in [57], we found SZ-related activity in the default mode network (DMN) when only analyzing DMN activity, whereas we had not previously found SZ-related activity in the DMN when analyzing whole-brain dFNC [75]. Examining less influential networks or dimensions may provide insights that previous analyses have overlooked.
This chapter presented an automatic pipeline based on feature learning to uncover network dynamics less influential to the initial clustering. We demonstrate the viability of our approach within the context of schizophrenia (SZ), applying our approach to a dataset consisting of 151 participants with SZ and 160 controls (HCs). We find that removing some connectivity pairs significantly affects the underlying states and magnifies the differences between participants with SZ and HCs in each state. Given our findings, we hope that our approach will contribute to the characterization and improved diagnosis of various neurological conditions and functions.

4.2 Materials and methods

4.2.1 Dataset

We used the Functional Imaging Biomedical Informatics Research Network (FBIRN) dataset of rs-fMRI recordings from 151 SZs and 160 HCs without any age and sex difference. The data was collected from 7 sites: the University of California at Irvine, the University of California at Los Angeles, the University of California at San Francisco, Duke University/the University of North Carolina at Chapel Hill, the University of New Mexico, the University of Iowa, and the University of Minnesota. Across all sites, participants provided written informed consent via processes approved by local institutional review boards.

4.2.2 Preprocessing and intrinsic component extraction

We used the same preprocessing as used in Chapter2. After preprocessing, we extracted independent components using the Neuromark pipeline in the GIFT toolbox http://trendcenter.org/software/gift. We identified 7 DMN subnodes: 3 Precuneus (PCu)
subnodes, 2 anterior cingulate cortex (ACC) subnodes, and 2 posterior cingulate cortex (PCC) subnodes (Table 2-1 and Step 1 in Figure 4-1B).

4.2.3 dFNC estimation

We used a sliding tapered window (window size = 20 TRs = 40 s) to estimate dFNC of seven subnodes in DMN for each individual. The resulting size of the FNC matrices is $7 \times 7$ due to the seven DMN subnodes (Step 2 in Figure 4-1B).

4.2.4 clustering

Then, we concatenated all dFNC values across all individuals and applied k-means clustering with $k=2$, Euclidian distance metrics, and 1000 iterations. Additionally, we estimated the state vector, a vector that represents the state of DMN at any given time (Step 3 in Figure 4-1B).

4.2.5 Feature learning approach

After clustering in each iteration, we used a feature learning approach to find the important feature that drives the clustering more than other global permutation percent change (G2PC) integrated with k-means clustering. G2PC is a form of the permutation feature learning method [79] that quantifies the sensitivity of clusters to feature perturbation by estimating the percentage of all samples that change from their original pre-permutation clusters to different post-permutation clusters. The percentage of samples that switch cluster following the permutation of a particular feature reflects the importance of that feature to the clustering. The features that result in the most significant number of samples to switch clusters following permutation are considered the most
important, and likewise, those features that do not cause samples to change clusters are deemed unimportant to the clustering.

In summary, our approach has multiple stages. 1) We assigned the dFNC samples to one of two clusters using the k-means algorithm. We arbitrarily decided upon the use of 2 clusters for ease of implementation, 2) We applied G2PC to the resulting clusters to identify the relative importance of each feature to the clustering, 3) After identifying feature
importance, we removed the most important feature, and 4) we iteratively repeated steps 2 through 4 until only one feature remained (Step4 in Figure 4-1B).

4.2.6 State change across iterations

After applying our feature learning approach, we examined the change in the 2 states across iterations. First, we visually aligned the clusters from each iteration. We then calculated the mean value along each dimension for the samples belonging to each subject in each of the two states. After calculating the mean values of each subject for each iteration, we computed the change in cluster center for each subject between each iteration. Because of the iterative reduction in the number of features, we computed the distance between the remaining features at each iteration (e.g., if iteration 1 has \( N \) features and iteration 2 has \( N-1 \) feature, we calculated the difference between each \( N-1 \) features). In this study, we used a weighted Euclidean distance to account for the decrease in the distance that accompanied the decrease in the dimensionality of the feature space. Specifically, we divided the Euclidean distance by the number of features at the iteration. Additionally, we used other distance metrics, including cosine and correlation.

4.2.7 dFNC features estimation

After identifying the dFNC states in each iteration, we estimated the proportional amount of time each participant spends in each state, called occupancy rate or OCR, and the number of between-state transitions or NOT.

4.2.8 Statistical analysis

We then performed statistical tests to identify differences between SZs and HCs similar to the tests described in the previous section. 1) We performed multiple comparisons, one-
way ANOVA tests to determine whether there were significant differences between the cluster centers, OCR, and NOT values at each iteration and the previous iteration when we combined SZs and HCs, 2) We performed two-sample t-tests to determine whether there were statistically significant differences between HCs and SZs on cluster centers, OCR, and NOT in each iteration, and 3) We ran a partial correlation between the OCR and NOT of SZs and symptom severity at each iteration while accounting for age, sex, and site.

4.3 Results

4.3.1 State changes across iterations

Figure 4-2 shows the cluster centroids of the two dFNC states across iterations, where obscured cells indicate connectivity pairs removed at a particular iteration. Figure 4-2A shows the change in distance (based on weighted Euclidian distance) between centroids for each subject in state 1 across iterations (red for HC and blue for SZ). Interestingly, while two PCu/PCu nodes were the first to be removed with our feature learning method, the ACC/PCC node in iteration 3 was the first to result in a significant change in distance between subject centroids across iterations. However, there was not an accompanying difference in the change between SZs and HCs.

Iterations 8 through 10, in which two PCC/PCu nodes and one PCu/PCu node were removed, were the first iterations in which there were differences in the subject centroids between classes. As the number of iterations increased, sensitivity to perturbation seemingly increased, as 14 (PCC/ACC), 15 (PCC/PCu), 17 (PCC/PCu), 19 (PCC/PCu),
and 20 (PCC/ACC) had increasing changes relative to the previous two iterations. Figure 4-2B shows the change in distance between centroids for each subject in state 2. State 2 had similar behavior to state 1 regarding when intra-subject centroid distances changed. However, states 1 and 2 had different iterations with significant differences between SZs and HCs. In state 2, differences in SZ and HC centroids occurred earlier in iteration 2 (PCu/PCu) and 3 (ACC/PCC) and less at later iterations. This indicates that more inter-class differences occurred in state 2 in earlier iterations following the removal of more influential modalities. However, more inter-class differences occurred in state 1 when less influential instances were removed.

4.3.2 Changes in temporal properties
Figure 4-3: Distance changes across iterations. A) State 1 across all iterations, B) State 1 across all iterations. The x-axis indicates the contribution of connectivity pair in the order of their removal from left to right. The y-axis indicates the distance changes.
Figure 4-4: dFNC feature changes across iterations. **A)** State 1 Occupancy Rate (OCR) for both healthy control (HC) and schizophrenia (SZ). Note that the OCR for state 2 is 1 minus the OCR for state 1. **B)** The number of between-state transition (NOT). Data for HCs and SZs are shown in red and blue, respectively. The x-axes show from left to right the node removed at each iteration, and the y-axis shows the occupancy rates. Asterisks indicate that there is a difference between values for SZs and HCs at a particular iteration.

**Figure 4-4A** shows the OCR of SZs and HCs for State 1. The removal of PCC/PCu, PCC/PCC, PCu/PCu, PCC/ACC, and PCC/ACC nodes at iterations 5, 7, 9, 11, and 20, respectively, corresponded to significant differences in occupancy rates for participants.
with SZ and HCs. In these cases, SZs spent significantly less time in State 1 and more time in State 2 relative to HCs. For the most part, these changes do not correspond to changes in the state across iterations, except in the case of iteration 9. However, class-related differences did not occur until more influential nodes were removed, which supports the overall utility of the method. Figure 4-4B shows the NOT of HCs and SZs in different iterations. As this figure shows, we do not observe a significant difference in the between-state transition in all iterations except iteration 13, in which we remove a feature representing connectivity between PCC and PCu.

4.3.3 Change in correlation with symptom severity

Figure 4-5 shows the link between OCR and NOT with symptom severity (both PANSS_P and PANSS_N) while controlling for age and sex in which iteration. This figure shows that we did not observe a significant link between dFNC features and symptom severity before removing any connectivity features. The first significant link between OCR and PANSS_P was observed in iteration 11, when we removed a PCu/PCC connectivity. Also, the first significant link between OCR and PANSS_N was observed in iteration 9, which
removed an AAC/PCu connectivity feature. In none of 20 iterations we observed a significant link between NOT and symptom severity.

4.4 Discussion

We hypostatize that the conventional dFNC framework potentially misses some dynamics in the FNC while some highly active regions mask the less active ones. We proved this idea in FBIRN dataset. We showed OCR and NOT estimated from DMN can not differentiate SZ from HC before removing highly influential connectivity features. However, after removing some connectivity features, we uncovered some hidden dynamics than can differentiate two groups based on OCR and NOT. This shows that our initial hypothesis was correct.

While here, we introduced a problem of the conventional method in analyzing the dFNC data and proposed a framework to solve this problem. It also can be used as a method as a biomarker of brain disorders. For example, in our study, we show the role of PCu/PCu connectivity feature (iterations 5 and 11) in differentiating between HC and SZ. To be precise, after we removed these connectivity features in iterations 5 and 11, we can differentiate the two groups. In other words, if PCC/PCu exits in our network, we cannot discriminate between HC and SZ.

4.4.1 Limitations and future work

In this study, we used G2PC as a feature importance metric. G2PC has some difficulty providing feature importance estimates for data in high-dimensional spaces. However, it should theoretically work well for the relatively low number of 21 DMN features we analyzed in this study. Future applications of our approach might examine other clustering
algorithms or interpretable clustering methods. We used a weighted L2 distance approach for quantifying the amount of change within states across iterations. We weighted the L2 metric to account for the natural decrease in distance between any two points within the feature space that would result from reduced dimensionality, and it is feasible that other weighting approaches or distance metrics could provide useful alternatives. Also, for the sake of simplicity in the initial application of our novel approach, we arbitrarily chose only to generate 2 clusters with k-means. Optimizing the number of clusters in the first iteration might be worthwhile in future work. Lastly, while we applied our approach to dFNC data from SZs and HCs, our approach is broadly applicable to dFNC and static FNC regardless of underlying conditions. In the future, we would like to apply our approach to larger, richer datasets containing information from participants with disorders beyond SZ.

4.4.2 Conclusion

This study presents a novel dFNC pipeline based on an unsupervised feature learning approach that provides unique insights into networks that existing dFNC analysis approaches may overlook. Existing dFNC analysis approaches often use clustering algorithms to identify substates of network activity. Those algorithms may be influenced more by the statistical qualities of the networks (e.g., variance) or by the presence of many dimensions than by condition-related activity. We apply our approach to a dataset consisting of 151 SZs and 160 HCs and find that the removal of some connectivity pairs significantly affects the underlying states and magnifies the differences between participants with SZ and HCs in each state. Our approach has the potential to contribute to the characterization of many neurological disorders and cognitive functions that could lead to the improved diagnosis and treatment of those disorders.
CHAPTER 5. VISUALIZING FUNCTIONAL NETWORK CONNECTIVITY DIFFERENCE BETWEEN HEALTHY CONTROL AND PATIENTS USING AN EXPLAINABLE MACHINE LEARNING METHOD

5.1 Introduction

In recent years, functional network connectivity (FNC) obtained from resting-state functional magnetic resonance imaging (fMRI) time series has revealed a great deal of knowledge about brain dysconnectivity in neurological and neuropsychiatric disorders and discriminating these patients from healthy subjects [17], [80]. A limited number of samples with highly dimensional FNC features makes the diagnosis process challenging. To overcome this problem, several machine learning-based classifications are used to classify patients from healthy control (HC) subjects based on FNC data [81]–[88]. However, none of them did give any information about the underlying mechanism of brain FNC affected by brain disorders.

Simple models, including linear models and logistic regression, can explain the underlying decision mechanism taken by the model in a prediction or classification problem. However, we need to sacrifice the model performance regarding classification accuracy. We usually get the best classification accuracy by using more complex models like random forests, decision trees, gradient boosted trees, and deep learning models. However, because of the nonlinear structure of the model, it is challenging to interpret the model. Therefore, there is always a trade-off between model interpretability and model accuracy in the classification task.
Recent development in explainable machine learning opens a new avenue to excavate the difference between the FNC of the healthy brain from the disease group [89]–[92]. In this chapter, by leveraging a machine learning approach and using whole-brain functional connectivity, we quantified the difference between patient and healthy subjects using an explainable Shapley Additive exPlanations (SHAP) approach [90], [91]. Through this feature learning method, we would be able to explain the model and find a subset of the most important features that contribute to the classification between patients and healthy participants.

5.2 Materials and methods

The proposed framework is shown in Figure 5-1. In this framework, we first preprocess rs-fMRI data and extract independent components for the whole brain. Next, we estimate the whole-brain FNC for each participant. Next, we classify between two classes of participants (e.g., patient vs. control). Finally, we use the SHAP method to extract a subset of features that contribute the most to the classification between two groups.

5.2.1 Dataset

This study used three datasets to validate the method.

5.2.1.1 Schizophrenia

The first dataset is from the Functional Imaging Biomedical Informatics Research Network (FBIRN) [93] projects. The FBIRN dataset includes seven sites containing 151 SZ subjects and 160 HC. The SZ group contains 115 males and 36 females, and the age mean, and standard deviation are 36.76 and 11.63, respectively. In the HC group, we have 115 males and 35 females; the mean and the standard deviation of the age is 37.03 and 10.86, respectively. A two-sample Kolmogorov-Smirnov test was used to show that the age and
sex difference between HC and SZ groups is not significant. The raw imaging data were collected from seven sites, including the University of California, Irvine; the University of California, Los Angeles; the University of California, San Francisco; Duke University/the University of North Carolina at Chapel Hill; the University of New Mexico; the University of Iowa; and the University of Minnesota. In this study, written informed consent was obtained from all participants. Institutional review boards approved the consent process of each study site [94].

Imaging data were collected at six of the seven sites using a 3T Siemens Tim Trio System and at one site using a 3T General Electric Discovery MR750 scanner. Resting-state fMRI scans were acquired using a standard gradient-echo echo-planar imaging paradigm: FOV of 220 × 220 mm (64 × 64 matrices), TR = 2 s, TE = 30 ms, FA = 770, 162 volumes, 32 sequential ascending axial slices of 4 mm thickness and 1 mm skip. Subjects had their eyes closed during the resting state scan [94].

5.2.1.2 Synthetic data
To test the reliability of the SHAP method, we generated synthetic data with the same number of features and samples as FBIRN. We first randomly generated 1378 features for each instance of both classes. In total, we generated 151 samples (to mimic SZ) and 160 samples to emulate HC groups. Then, we increased the value of some features for one class and decreased those values for another class. Therefore, two blocks of features are significantly different between the two classes (see Figure 5-3).
The data is from healthy adult individuals with European ancestry available in the UK Biobank [95]–[97], which includes 9394 subjects (average age: 63; range: 45-81 years; 4783/4611: female/male). We used a median split to put all subjects into two old or OA (>63) and middle adult or MA (<63) groups. The middle adult group includes 4428 subjects (2406/2022: female/male), and the old group includes 4966 subjects (2377/2589: female/male). The mean age of the MA and OA groups is 55.96± 4.23 and 68.45± 3.66, respectively. Standard Siemens Skyra 3T with a standard Siemens 32-channel RF receiver head coil is used for neuroimaging data acquisition. High resolution T2*-weighted functional images were acquired using a gradient-echo EPI sequence with TE =39 ms, TR = 0.735 s, flip angle = 52°, slice thickness = 3.5 mm, slice gap = 1.05 mm, field of view: 88×88×64 matrix s, voxel size = 2.4 mm 2.4 mm 2.4 mm, and 6:00 min.

5.2.2 Preprocessing and intrinsic component extraction
The same preprocessing as described in Chapter 2 was used here. In the next step, to extract reliable, independent components (ICs), we used the Neuromark automatic ICA pipeline as introduced in [98]. We first identified the replicable components in the pipeline by matching group-level spatial maps from two large-sample healthy control datasets. Then, a subset of matched components was identified as meaningful if they exhibited peak activations in the gray matter; had low spatial overlap with known vascular, ventricular, motion, and susceptibility artifacts; and had dominant low-frequency fluctuations on their time-courses. Then, we categorized ICN into seven domains, including subcortical network (SCN), auditory network (ADN), sensorimotor network (SMN), visual network (VSN), cognitive control network (CCN), default-mode network (DMN), and cerebellar network (CBN) based on anatomy and prior knowledge. In total, we extracted 53 ICs for the whole brain. 

**Figure 2-2** and **Table 2-1** showed all seven networks identified by Neuromark.

5.2.3 **Functional network connectivity**

To estimate the communication strength or FNC in the brain, we calculated the Pearson correlation between any pair of ICNs in each subject, as shown below

$$R = \frac{\sum_{n=1}^{N}(x_1 - \overline{x_1})(x_2 - \overline{x_2})}{\sqrt{\sum_{n=1}^{N}(x_1 - \overline{x_1})^2} \sqrt{\sum_{n=1}^{N}(x_2 - \overline{x_2})^2}}$$ 5.1

where $x_1$ and $x_2$ are timecourse signals and $\overline{x_1}$ and $\overline{x_2}$ are the mean of $x_1$ and $x_2$, respectively. It takes values in the interval $[-1, 1]$ and measures the strength of the linear relationship between $x_1$ and $x_2$. Each FNC is a $53 \times 53$ matrix, and with 53 ICs, we calculated 1378 connectivity features. The average FNCs across all MA and OA in UK Biobank and HC and SZ subjects in FBRIN are shown in **Figure 5-2A** and **Figure 5-2B**,
respectively. In this figure, the hot and cold colors represent positive and negative connectivity, respectively. Also, Figure 5-3 shows the FNC of the synthetic dataset.

5.2.4 Classification

5.2.4.1 FBIRN dataset (SZ vs. HC)
Figure 5-3: Functional network connectivity (FNC) in the synthetic dataset. 
A) The average FNC across all samples of Class1 and Class2. The color bar shows the intensity of the correlation. The size of the connectivity matrix is 53×53, then in total, we have 1378 connectivity features for each sample. We first randomly generate 1378 features for each sample of both classes. In total, we generated 151 samples (to mimic HC) and 160 samples to mimic HC and SZ groups in FBIRN dataset, respectively. Then, we increased the value of some features for one class and decreased those values for another class, 
B) A zoomed version of the difference between two classes. The number is assigned to each feature is shown.
To classify SZ and HC subjects, we trained a convolutional neural network (CNN) and two decision tree-based classifiers, including random forest (RF) and XGboost (XGB), based on the FNC features. RF is one of the most popular ensemble tree-based learning algorithms that randomly select a subset of the training data. Then, it collects the vote from a different decision tree to assign a class to the test data. In RF, we trained a model with 200 trees. In this model, three hyperparameters were optimized via internal cross-validation, including the maximum depth level of 90, the minimum sample split of 5, and the minimum sample points of 2 at each node. For the other hyperparameters, we used the default values in scikit-learn. XGB is another popular ensemble decision tree learning algorithm that builds the trees sequentially by minimizing the error of the previous tree. In other words, each tree updates the residual errors by learning from its predecessors in this method. In this study, the learning rate, the number of trees, gamma, the maximum depth level, subsample ratio were set to be 0.07, 2800, 16, 3, and 0.8, respectively. We used the default values in scikit-learn for the other hyperparameters.

The CNN contains two 2D-convolution layers with ReLU activation functions followed by a max-pooling layer, dropout, a fully connected layer with L2-normalization, and another fully connected layer with a softmax activation function. The number of hidden layers, dropout rate, regularization parameter, and learning rate were optimized through a grid search. The dropout rate was optimized to be 0.4, and the weight decay value for $L_2$ normalization was set to be 0.001. Adam, with a learning rate of 0.001, was used for optimization. In addition to dropout and regularization, early stopping was utilized for CNN to prevent overfitting by monitoring validation loss.
5.2.4.2 **UK Biobank dataset (MA vs OA)**

We trained three different tree-based classifiers to classify middle adults from old, including random forest (RF), XGB, and CATBoost (CAT). For RF, we trained a model with 1800 trees, the maximum depth level of 34, the minimum sample split of 2, and the minimum sample points of 4 at each node. For XGB as a tree booster, learning rate, the number of trees, gamma, the maximum depth level, and subsample ratio was set to be 0.01, 1800, 10, 8, and 1, respectively. The CAT has the learning depth and learning rates of 4 and 9, respectively. We iterated this process 300 times.

5.2.4.3 **Synthetic dataset (Class1 vs. Class2)**

Like FBIRN dataset, we trained an RF, XGB, and CCN to classify Class1 from Class2 of the synthetic dataset.

5.2.4.4 **Classifier evaluation**

In all classifiers, we used a 10-fold classifier. We calculated the area under ROC (AUC), accuracy, sensitivity, specificity, and F1 score to assess the classifier's performance, as shown in the equations below.

\[
\text{Accuracy} = \frac{TP + TN}{TP + FN + TN + FP} \quad 5.2
\]

\[
\text{Sensitivity} = \frac{TP}{TP + FN} \quad 5.3
\]

\[
\text{Specificity} = \frac{TN}{TN + FP} \quad 5.4
\]

\[
F_1 = \frac{2TP}{2TP + FP + FN} \quad 5.5
\]

where TP, FN, TN, and FP denoted true positive, false negative, true negative, and false positive, respectively.
5.2.5 *SHapley Additive exPlanations (SHAP)*

To explain the difference between two classes in the trained models, we leveraged the SHAP method, which ranks the contribution of each feature based on its Shapley value from coalitional game theory. In the game theory, two or more players (or features) are working together but with an unequal contribution to achieving the desired outcome. The Shapely value reasonably estimates the contribution of both gains and costs of several players working in a coalition. The Shapley value estimates the magnitude of each feature contribution as the feature importance and estimates the direction (or the sign) of a feature [99]. The positive sign shows the activity, whereas the negative sign shows the inactivity of a specific feature in the model. In this approach, for a given model, i.e., $f(x)$, we computed the Shapley values using a weighted sum that characterizes the impact of each feature being added to the model-averaged over all possible orders of features being introduced:

$$
\varnothing_{i,j} = \sum_{S \subseteq \{i,j\}} \frac{|S|!(M-|S|-2)!}{2!(M-1)!} \delta_{i,j}(S)
$$

when $i \neq j$ and:

$$
\delta_{i,j}(S) = f_x(S \cup \{i,j\}) - f_x(S \cup \{i\}) - f_x(S \cup \{j\}) + f_x(S)
$$

where $S$ denotes all possible feature coalitions, $M$ denotes the number of all features used in the model. The contribution or Shapley value (or $\varnothing_{i,j}$) of each set of features, i.e., $i$ and $j$ here, is determined by averaging their contribution across all possible permutations of a feature set [90], [91].
5.3 Results

5.3.1 Classification results between SZ/HC and MA/OA

Figure 5-4A shows the classifier ROCs for RF (green), XGB (blue), and CNN (red) in the classification between SZ and HC in the FBIRN dataset. In addition, Table 5-1 shows the mean accuracy, mean F1, mean sensitivity, and mean specificity of these classifiers. CNN significantly outperforms the other two decision tree-based classifier based on their 10-fold classification results (corrected $p<0.05$). Also, Figure 5-4B shows the classifier ROCs for RF (green), XGB (blue), and CAT (red) in the classification between MA and OA in UK Biobank dataset.
Table 5-1 Performance of different classifiers in SZ/HC classification with 10-fold cross-validation

<table>
<thead>
<tr>
<th></th>
<th>Mean Accuracy</th>
<th>Mean AUC</th>
<th>Mean F1</th>
<th>Mean Sensitivity</th>
<th>Mean Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>RF</td>
<td>0.76</td>
<td>0.82</td>
<td>0.76</td>
<td>0.77</td>
<td>0.77</td>
</tr>
<tr>
<td>XGB</td>
<td>0.76</td>
<td>0.81</td>
<td>0.76</td>
<td>0.78</td>
<td>0.78</td>
</tr>
<tr>
<td>CNN</td>
<td>0.88</td>
<td>0.88</td>
<td>0.88</td>
<td>0.90</td>
<td>0.84</td>
</tr>
</tbody>
</table>

Table 5-2 Performance of different classifiers in MA/OA classification with 10-fold cross-validation

<table>
<thead>
<tr>
<th></th>
<th>Mean Accuracy</th>
<th>Mean AUC</th>
<th>Mean F1</th>
<th>Mean Sensitivity</th>
<th>Mean Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>RF</td>
<td>0.65</td>
<td>0.71</td>
<td>0.70</td>
<td>0.65</td>
<td>0.67</td>
</tr>
<tr>
<td>XGB</td>
<td>0.68</td>
<td>0.75</td>
<td>0.71</td>
<td>0.68</td>
<td>0.68</td>
</tr>
<tr>
<td>CAT</td>
<td>0.68</td>
<td>0.75</td>
<td>0.70</td>
<td>0.69</td>
<td>0.67</td>
</tr>
</tbody>
</table>

Additionally, Table 5-2 shows the mean accuracy, mean F1, mean sensitivity, and mean specificity of MA/OA classification. Overall, we did not observe a significant difference in the classifier's performance. However, GXB showed higher AUC values among all classifiers.

5.3.2 SHAP result in the classification between SZ and HC

The main focus of the current study is finding the underlying machine of the difference between SZ and HC subjects using an explainable machine learning approach. We found a subset of connectivity features that contribute most to RF, XGB, and CNN models using the SHAP method. Figure 5-5 shows the top 20 connectivity features, which contributed more than the others in classifying SZ and HC subjects for all three modes in descending order. In these graphs, the red color shows an increase in the connectivity feature, and the blue color shows a decrease in the connectivity. The positive and negative Shapley value corresponds to SZ and HC groups, respectively. In these three graphs, those connectivity features are selected only by RF, and XGB is shown in red. Those connectivity features
overlapped between RF and CNN are shown in orange, and those chosen in both XGB and...
CNN are shown in blue. Finally, those connectivity features are selected from SHAP and overlapped across all three models shown in purple.

**Figure 5-5A** shows that the subcortical network contributed to 14 out of the top 20 features in the RF model. The next brain network that has the most contribution to the classification between SZ and HC in the RF model is the VSN. Also, we observed that all brain networks contributed to the top 20 features selected by the SHAP in this model. A disrupted pattern was observed in the connectivity between the subcortical and other brain networks. In 9 connectivity features related to the SCN, increasing the connectivity would increase the likelihood of the HC group in the classifier's output while increasing connectivity strength in the other 5 connectivity features related to the SCN, put the classifier output at the SZ group.

Interestingly, in all connectivity features related to VSN, increasing the connectivity strength increases the likelihood of the HC group at the output of the RF classifier. In addition, we found that the connectivity between the VSN and the SCN is higher for HC subjects. Overall, we observed a disrupted pattern in the top 20 connectivity features selected by SHAP in the RF model.

**Figure 5-5B** shows the top 20 connectivity features selected by SHAP in XGBoost in the classification between HC and SZ subjects. Like RF, we observed that the subcortical network contributes to the top 20 features selected by SHAP. The second network that contributes most to this model is the cognitive control network. Also, we found a disrupted (i.e., both increase and decrease) pattern in subcortical network connectivity in HC vs. SZ subjects. Also, similar to the RF model, the connectivity between subcortical and visual
networks is more for the HC subjects. Similar to RF, we observed a disrupted pattern in
the connectivity of the top 20 features in this model.

**Figure 5-5C** shows the top 20 connectivity selected by SHAP in the CNN model. Again,
similar to the other two models, we found that the subcortical network contributed more
than the other networks in classifying HC and SZ subjects. The cognitive control network
is the second-highest network in this model regarding the contribution. We found similar
patterns in this model as we observed in RF and XGB. For example, increasing the
connectivity between subcortical and visual networks increases the likelihood of the HC
group at the classifier's output. We also observed a disrupted pattern in the connectivity
related to subcortical networks similar to RF and XGBoost. **Figure 5-6A, Figure 5-6B, and
Figure 5-6C** visualize the connectivity difference between HC and SZ subjects selected by

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**Figure 5-6: Visualization of top 20 features selected by SHAP in three models used in the FBIRN dataset.** A) Features selected by SHAP in the RF model, B) Features selected by SHAP in the XGB model, C) Feature selected by SHAP in CAT. Each line represents the connectivity between a pair of components. The blue line shows the higher connectivity in HC, and the red shows the higher connectivity in the SZ. All networks contribute to the top 20 features selected by the SHAP method. Also, in all three models, CCN and SCN have the higher contribution. Also, we observed both an increase and decrease in the difference between SZ and HC, which proved a disrupted pattern in brain connectivity in SZ.
SHAP in RF, XGB, and CCN, respectively. Each line represents the connectivity between a pair of components. Blue lines show higher connectivity in HC, and red lines show higher connectivity in the SZ. All networks contribute to the top 20 features selected by the SHAP method. Also, CCN and SCN have a higher contribution in all three models. Also, we observed both an increase and decrease in the difference between SZ and HC, which proved a disrupted pattern in brain connectivity in SZ.

5.3.3 SHAP result in the classification between MA and OA

Figure 5-7A shows the top 20 connectivity features which contributed most to the classification between MA and OA in the RF model. As this figure shows, CCN connectivity with the rest of the brain contributed to 13 connectivity features out of the top 20 features selected by SHAP in the classification between OA and MA subjects. SCN is the second network that contributed second-most in the top 20 features selected by the SHAP.

Figure 5-7B and Figure 5-7C showed that the top 20 connectivity features were selected by the SHAP method in the XGB and CAT models, respectively. In both models, SCN and CCN had the most contribution in classifying old and middle adult subjects. In addition, those connectivity features selected by the SHAP method in all three models are shown in purple. Those features only in RF and XGB are shown in green. Also, those connectivity features that are only selected in RF and CAT, in XGB and CAT, are shown in dark blue and orange, respectively. We see in these three graphs that we found 5 overlaps out of the top 20 features selected by SHAP in RF, XGB, and CAT. Also, we found that 9 features out of the top 20 features overlapped in the model of RF and XGB. We found 6 overlaps
Finally, we found 10 overlaps in the top 20 features in RF and CAT. Figure 5-7: SHAP feature selection results in the classification between MA and OA in UK Biobank dataset. A) Top 20 connectivity features (out of 1378 connectivity features) of the RF model selected by the SHAP method. B) Top 20 connectivity features of XGB selected by SHAP method. C) Top 20 connectivity features selected by SHAP method in CAT classifier. Also, in all graphs light, blue shows decreasing the connectivity feature and red shows increasing the connectivity features of RF classifier.
5.3.4 SHAP results on the synthetic data

We trained three classifiers, including RF, XGB, and CNN, based on the connectivity features from the synthetic dataset and then applied the SHAP method to the model.

Figure 5-9 shows the top 20 features selected by the SHAP method in the synthetic dataset. In these graphs, higher SHAP values represent Class 1, and lower SHAP values represent Class 2. For example, in increasing feature 1225, put the RF classifier output at Class 1, and decreasing this feature, put the RF classifier output at Class 2. The increase and decrease patterns are completely matched with what we see in Figure 5-3. These results verify that the SHAP method can completely capture the difference between the two features selected by SHAP in XGB and CAT.
classes. We found that two out of the top 20 features overlap among all three models, as

Figure 5-9 SHAP feature selection results in the synthetic dataset: A) Top 20 connectivity features (out of 1378 connectivity features) of RF model selected by SHAP method, B) Top 20 connectivity features of XGB selected by SHAP method, C) Top 20 connectivity features selected by SHAP method in CCN classifier. Also, in all graphs, the light blue shows decreasing the connectivity feature, and red shows increasing the connectivity features. For example, the first connectivity feature selected by the SHAP method in Random Forest is feature #1225, in which increasing (red) this connectivity feature would increase the likelihood of Class2 at the output of RF and decreasing (light blue) this connectivity would increase the likelihood of Class2 at the output of RF classifier.
shown by purple in Figure 5-9.

5.4 Discussion

In the current study, we developed a pipeline based on the SHAP method to identify the functional network connectivity, estimated from rs-fMRI, biomarkers to differentiate two groups of subjects. We validated this framework on a synthetic dataset and two publicly available datasets, including FBIRN and UK Biobank dataset. Using the synthetic dataset, we validated the proposed framework on three models. In all models, the framework only selected those features that are different between two classes of the synthetic data.

5.4.1 Schizophrenia biomarker

The current study explored the functional connectivity among several data-driven networks, including SCN, ADN, VSN, SMN, CCN, DMN, and CBN, to differentiate individuals with schizophrenia from control. We trained three models, including RF, XGB, and CCN. We found that all three models can successfully differentiate these two groups in which CCN showed the highest classification accuracy.

Next, we estimated the top 20 features out of 1378 that showed the most contribution in classifying SZ and HC. We found that three features out of the top 20 selected features overlap across all three models, while 9 features overlap between RF and XGB, 5 features overlap between RF and CCN, and 5 features overlap between XGB and CCN. This amount of overlap on the top 20 features selected by SHAP in three different models might prove the robustness of the proposed framework.

We found that all seven networks significantly contributed to discrimination between individuals with schizophrenia from healthy people. In oppose to the main body of previous research, which only focused on the functional connectivity of DMN in schizophrenia [100],
we found that functional connectivity of other connectivity, in particular CCN and SCN, contributes to the discrimination between SZs and HC.

5.4.2 The aging biomarker

The current study explored the functional connectivity among several data-driven networks, including SCN, ADN, VSN, SMN, CCN, DMN, and CBN. Then the differences in connectivity network feature among these networks between older adult (≥ 63) or OA and middle adult (<63) or MA subject using rs-fMRI of the UKBiobank dataset. We trained and tested three tree-based models, including RF, XGBoost, and CAT. Using three models, we could successfully differentiate OA from MA subjects with a mean accuracy of more than %65, while XGB showed the highest accuracy, around %68. This result highlighted the role of the brain FNC in the classification of old and middle adult subjects.

In the next step, we found a subset of features that have the highest contribution to the classification between OA and MA subjects in each model. We discovered that %25 of the top 20 features were shared among all three models. We also found that all seven networks, including SCN, ADN, SMN, VSN, CCN, DMN, and CBN, contributed to all top 20 connectivity features selected by the SHAP method in all three models. This is consistent with previous studies, which showed the effect of age on the between-network connectivity in the adult subjects [101], [102]. In contrast to the study mentioned above, in which the statistical learning method was used to find the difference in the FNC between the OA and MA subjects, in the current study, we used a novel feature learning method to model this difference. In opposition to statistical learning, which typically evaluates each feature one by one and does not consider the interaction between input features, the machine learning-
based feature learning approach provides a generalized model of the difference between the older adult and middle adult features [103].

By comparing the connectivity between OA and MA subjects in the connectivity of the top 20 connectivity features, we found a pattern of both increases and decreases in this connectivity. That possibly showed a disrupted pattern in the whole-brain FNC, consistent with the previous literature [104]. We finally found that CCN and SCN contribute more than the other network in this classification, and the result of consistent among all models. This is consistent with previous literature, where the role of CCN and SCN was highlighted [105], [106].

5.4.3 Comparing SHAP with other feature selection method

There are a few advantages of using SHAP over the other interpretable approaches. Since SHAP values describe importance and contributions as the sum of the feature contributions to an outcome, it produces a model-agnostic way to explain the model. In other words, it can be applied to any machine learning and deep learning model. At the same time, some other methods like LASSO (L1 norm) or elastic net (L1 and L2 norms) are restricted to a specific model. Additionally, LASSO for $n \ll p$ ($n$ is the number of samples and $p$ is the number of features), LASSO selects at most $n$ features [107]. Additionally, LASSO will use random selection of only one feature from a group of highly correlated features. While SHAP will find top features across the whole feature set and does not focus on the small regions in the feature space. Also, SHAP values show the feature's importance and indicate whether the feature has a positive or negative impact on the classifier output.

5.4.4 Limitations and future work
There are a few limitations associated with this study. First, we only used the SHAP method as an interpretable approach to different two groups of samples based on the whole-brain FNC. Therefore, future work is needed to explore other explainable methods and compare their result with SHAP. Second, the performance of other linear and nonlinear machine learning models should be evaluated in the classification between two groups based on the FNC.

5.4.5 Conclusion

This chapter proposed a framework to identify the FNC biomarkers through the SHAP method that differentiates two groups of samples. We validate the framework’s robustness in three datasets, including a synthetic dataset, FBRIN, and UK Biobank. While we proved that the framework finds only those FNC features different from two classes of the synthetic dataset, we found the FNC biomarker associated with schizophrenia and aging.
PART 2: THE CLINICAL IMPLICATIONS OF DFNC
CHAPTER 6. ALZHEIMER’S DISEASE PROJECTION
FROM NORMAL TO MILD DEMENTIA REFLECTED IN
DYNAMIC FUNCTIONAL NETWORK CONNECTIVITY

6.1 Introduction

Alzheimer’s disease (AD) is the most common age-related dementia, typically affecting individuals over 65 years of age [31]. AD usually progresses slowly in several stages, including mild (early stage), moderate (middle stage), and severe (late stage) [32]. To date, there is no way to cure AD, but some medications can decelerate its progress [108]. Therefore, predicting the progression from a normal stage to mild cognitive impairment and further to AD itself is an important step toward early medical intervention.

Resting-state functional magnetic resonance imaging (rs-fMRI) that indirectly measures neural processing in the brain based on the blood oxygenation can be used to identify spatially distributed networks in the brain. In recent years, functional connectivity or its network analog functional network connectivity (FNC), including dynamic FNC and static FNC, achieved from rs-fMRI time series has uncovered a great deal of knowledge about the brain dysconnectivity in various neurological disorder including schizophrenia [75], [109], major depression disorder [27], autism[110]–[112], ADHD [113], and AD [114]. In particular for AD, previous studies reported a reduction in the default-mode network FNC in AD compared with mild cognitive impairment (MCI) patients and healthy subjects [115]. Another study reported a difference in the FNC of sensorimotor network, visual network, and default mode network of healthy control (HC) subjects and AD patients [116].
By assuming that FNC is invariant or static over time, many of the AD-related studies mentioned above have focused on sFNC and ignored dFNC. Indeed, unlike conventional sFNC, which is obtained from the correlation within an entire time series, dFNC refers to the connectivity between any pair of brain networks within sub-intervals of time series [117]. In fact, dFNC research suggests that cognitive deficits and clinical symptoms associated with many neurological disorders do not only depend on the strength of the connectivity between any pair of brain regions but also on the variation of connectivity strength of those regions over time [19], [27], [117]. In recent years, a few papers studied dFNC in AD. For instance, we investigated whole-brain dFNC in AD and subcortical ischemic vascular disease (SIVD)[118]. Another study explored the temporal properties of dFNC associated with dementia in Parkinson’s disease [119]. However, the longitudinal dFNC changes from cognitively normal to mildly then severely cognitively impaired have not been extensively explored.

In this chapter, we explored the temporal dynamics of the whole-brain FNC from 1385 rs-fMRI scans of HC and very mild AD (vmAD). We used a sliding window approach followed by the k-means clustering method to identify a set of connectivity states [117]. Next, we calculated between-state transition probability via the hidden Markov model (HMM) and the amount of time each subject spends in a state, called occupancy rate or OCR, to model the temporal properties of dFNC. We investigated the correlation between HMM and OCR features with the clinical dementia rating scale sum of boxes (CDR-SOB) scores. In addition, we explored the link between state-specific connectivity features with CDR-
Finally, we trained a support vector machine (SVM) to predict from HC to vmAD based on the sFNC connectivity features and dFNC features, including HMM and OCR.

## 6.2 Materials and methods

### 6.2.1 Participants

In this study, the data we used are from the longitudinal Open Access Series of Imaging Studies (OASIS)-3 cohort, which was collected from several ongoing studies in the Washington University Knight Alzheimer Disease Research Center over 15 years [113]. This data contains 1385 rs-fMRI imaging and related clinical and demographic data at the time of scanning (from 910 subjects) with ages ranging from 42 to 95 years. For each subject, the imaging data, demographic, and clinical dementia rating (CDR) scale were used in any stage of cognitive functionality. All participants must have CDR≤1 at the time of the clinical core assessment, and once the participant reached CDR=2 or CDR-SOB>9, they were no longer eligible for the study [113]. We evaluated the cognitive stage of the participants at the time of the scanning based on the clinical dementia rating scale sum of boxes (CDR-SOB) scores and organized them into 2 groups, including healthy control or HC (CDR-SOB=0), very mild AD or vmAD (0.5≤CDR-SOB≤9) [120]. In total, we have 1028

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<td></td>
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<tr>
<td>Age</td>
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<tr>
<td>CDR-SOB</td>
<td>0±0</td>
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<tr>
<td>Gender (M/F)</td>
<td>215/142</td>
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<tr>
<td>Age</td>
<td>75.10±7.85</td>
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<tr>
<td>CDR-SOB</td>
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Note: HC: Healthy control; vmAD: very mild Alzheimer Disease; M:male; F:female; CDR-SOB: clinical dementia rating scale sum of boxes
scan of HC, 357 scans of vmAD patients. The demographic information is provided in Table 6-1.

6.2.2 Imaging data acquisition

Two Trio 3T with a 20-channel head coil were used to collect imaging data (Siemens Medical Solutions USA, Inc). High resolution T2*-weighted functional images were obtained by an echoplanar imaging or EP sequence with TE =27 ms, TR = 2.5 s, flip angle

Figure 6-1: Analytic pipeline. Step1: The time-course signal of 53 ICNs has been identified using group-ICA in the Neuromak template. Step2: After identifying 53 ICNs, a taper sliding window was used to segment the time-course signals and then calculated the functional network connectivity (FNC). Each subject has 139 FNCs with a size of 53×53. Also, we calculated static FNC for the entire time of recording. Step3: After vectorizing the FNC matrixes, we concatenated them, and then a k-means clustering with correlation as distance metrics was used to group FNCs into three distinct clusters. Step4: Then, based on the state vector, we calculated between-state transition probability or hidden Markov model (HMM) features and occupancy rate (OCR) for each subject. In total, nine HMM features and three OCR were estimated from the state vector of each subject. Step5: To find a link between FNC features, including sFNC and dFNC feature with clinical dementia rating scale sum of boxes (CDR-SOB), we used partial correlation by accounting for age and gender.
= 90˚, slice thickness = 4mm, slice gap = 4 mm, matrix size = 64, and voxel size of 1 mm × 1 mm × 1.25 mm. The duration of the scanning was 6 minutes.

6.2.3 Preprocessing

We used the same pre-processing and independent components (ICs) extraction introduced in Chapter 2 to extract 53 ICs for the whole brain (Step 1 of Figure 6-1).

6.2.4 Functional network connectivity

The static functional network connectivity (sFNC) of each subject was calculated by computing the Pearson correlation between any pair of ICNs time series. With 53 ICNs, it resulted in 1378 whole-brain correlation values for each subject. In addition, for each subject \(i = 1 \ldots N\), the dynamic FNC (dFNC) of the whole brain was estimated via a sliding window approach. A tapered window was obtained by convolving a rectangle (window size = 20 TRs = 50 s) with a Gaussian (\(\sigma = 3\)) was used to localize the dataset at each time point. It is worth mentioning that previous studies suggested that a window size between 30-60s is a reasonable choice for capturing the dFNC fluctuation [121]. Based on this past work, we used the 50s as the window size. A covariance matrix, based on Pearson correlation, was calculated to measure the dFNC between ICs. The dFNC estimates of each window for each subject were concatenated to form a \((C \times C \times T)\) array (where \(C=53\) denotes the number of ICNs and \(T=139\)), which represented the changes in brain connectivity between ICNs as a function of time (Step 2 of Figure 6-1) [117].

6.2.5 Clustering and dFNC latent features

We used k-means clustering to partition dFNC window into a set of separated clusters (states). Based on the elbow criterion (the ratio of within to between cluster distance), we found that the optimal number of clusters (i.e., \(k\)) is 3. We used correlation based on
Pearson correlation as a distance metric in the clustering algorithm in 1000 iterations [4], [117] (Step3 of Figure 6-1). The output of this step is 3 states for all subjects and a subject-specific state vector. The state vector shows the state of the whole-brain FNC of each subject at a specific time. In the next step, we calculated the between-state transition probability based on HMM. The transition probability, $a_{ij}$, is the probability of the system to transition from state $j$ at time $t$ to state $i$ at time $t+1$.

$$a_{ij} = p(s(t + 1) = i|s(t) = j) \quad 6.1$$

In addition, we computed the OCR of dFNCs in each state (Step4 of Figure 6-1). In addition, for each subject, we averaged all dFNC belonging to a state as her/his state-specific FNC. In more detail, each subject has multiple dFNC in each state. Then, in each state, we used the average of dFNC (i.e., the average of 1378 connectivity features) of each subject as her/his state-specific FNC.

6.2.6 Statistical analysis

To assess the link between dFNC features, including state-specific FNC, OCR, and HMM with CDR-SOB, we used partial correlation by accounting for age and gender. We performed statistical analysis on all 1378 whole-brain connectivity features, 9 HMM features, and 3 OCR features, separately (Step5 of Figure 6-1). All $p$ values have been adjusted by the Benjamini-Hochberg correction method for multiple comparisons[73]. The number of the null hypothesis in state-specific FNC, OCR, and HMM were 1378, 3, and 9, respectively.

6.2.7 Dementia progression is associated with functional network connectivity
In the next step, we explored whether functional network connectivity, including sFNC and dFNC features, can predict the progression of AD. We put subjects into two different groups. The first group contains those subjects who remained in HC, whom we call the unconverted HC or uc-HC stage within the next five years of the first scan, and the second group contains those subjects whose cognitive functionality changed from HC to vmAD (0.5 ≤ CDR-SOB ≤ 9), and we call c-HC. The first group contains 85 subjects (48 females and 37 males) with a mean age of 74.6478 and a range between 65 and 85. The second group contains 40 subjects (18 females and 22 males) of which the mean age is 74.6878, and the range of the age is between 64 and 85. Using a two-sample t-test, we did not observe a significant difference between the age and gender of these two groups (t(123) = -0.90, p=0.36). We trained a support vector machine (SVM) based on sFNC, OCR, and HMM features from the baseline rs-fMRI (around five years prior to the conversion) to differentiate these two groups.

One major problem in this classification is imbalanced datasets. To deal with this problem, we have used a data augmentation method called the adaptive synthetic (ADASYN) sampling approach. In this method, we adaptively generated synthetic data for the minority class based on the distribution of both classes. ADASYN generates synthetic data for the part of the minority class that is harder to learn than those minority samples, which are easier to learn. In this study, we have a dataset with 85 samples in major class and 40 samples in minor class. Using ADASYN, we generated 45 samples of synthetic data for the minor class to make the dataset balanced [122]. We trained an SVM with a polynomial kernel function, as shown in Equation 6.2) to classify two classes [123].

\[ k(x_1, x_2) = (1 + x_1'^Tx_2)^p \] 6.3
where \( p \) is a positive integer value.

There are a few advantages of using SVM. 1) SVM works well for high-dimensional data. 2) SVM is effective when the number of samples is smaller than the number of dimensions, which is a common problem for neuroimaging data. 3) SVM can handle nonlinearity in the data using a kernel trick. This provides an advantage over linear classifiers like logistic regression. However, choosing the appropriate kernel function is not easy. In addition, due to the limited number of samples, it is not advisable to use a neural network classifier. It is worth mentioning that an imbalanced dataset is a challenging problem in SVM classification [124]. Our study used ADASYN to generate synthetic data of the minatory class to elevate this problem. In more detail, we used the ADASYN-based sample and a subset of the major class (i.e., uc-HC, \( N=45 \), the ADASYN sample size) and trained a model, and then we tested that model on real unseen data from both uc-HC and c-HC groups. We iterate this 10 times. This number was chosen arbitrarily. It is worth mentioning that changing the number of iterations would not change the classification result. In each iteration, we used the 5-fold cross-validation method in which we used 80% of the training data to train a model and 20% of the data to validate that. The hyperparameters of the SVM classifier were selected through an optimization process.

We calculated the classification accuracy, sensitivity, specificity, and area under the receiver operating characteristic curve (AUC) to assess the classification performance. Accuracy, sensitivity, and specificity were quantified by:

\[
\text{Accuracy} = \frac{TP + TN}{TP + FN + TN + FP}
\]

\[
\text{Sensitivity} = \frac{TP}{TP + FN}
\]
\[ \text{Specificity} = \frac{TN}{TN + FP} \]

where TP, FN, TN, and FP denoted the number of uc-HC subjects correctly predicted, the number of c-HC subjects classified as uc-HC subjects, the number of uc-HC correctly predicted, and the number of un-HC subjects classified as c-HC subjects, respectively.

6.3 Results

6.3.1 Dynamic functional connectivity states

Figure 6-2 shows the reoccurring connectivity states identified by the k-means clustering method. In all states, we observed strong positive connectivity within SMN and VSN, and CBN. State 3 showed the strongest connectivity within SMN and within VSN among all states. In addition, this state had the highest connectivity between SMN and VSN. Also, this state was separated from other states by showing the lowest negative connectivity between SMN and VSN with the rest of the brain. State 1 showed the lowest connectivity between SMN and VSN. Finally, we measured the OCR of each subject in state 1, state 2, and state 3. OCR represents the amount of time each subject spends in each state. Results showed that subjects spent an average of 23.78 %, 52.17 %, and 24.05 % in state 1, state 2, and state 3, respectively.

6.3.2 The correlation between state-specific FNC and CDR-SOB

Figure 6-3 showed the partial correlation between state-specific FNC and CDR-SOB while we controlled for age and gender. The significant correlations (uncorrected \( p < 0.05 \)) are shown in red (positive correlation) and blue (negative correlation). Also, a significant correlation that passes the multiple comparisons is marked by asterisks.
In state 1, we observed a significant and negative correlation between within-SCN, within-SMN, within-VSN connectivity, and CDR-SOB. This means that this connectivity decreases by progression from normal to mild dementia states. In this state, we found a reorganized (i.e., both positive and negative) pattern in the correlation between within-DMN connectivity and within CCN-connectivity with CDR-SOB. A similar reorganized correlation pattern was observed between the between-network connection and CDR-SOB. Specifically, a more reorganized pattern was observed in the correlation between SCN connectivity with the rest of the brain.

In state 2, similar to state 1, we observed a negative correlation between within-SCN, within-SMN, within-VSN, and within-CBN connectivity with CDR-SOB. We also observed both positive and negative correlations between the connectivity of CCN and DMN, and CDR-SOB. Compared with the other states, this state showed a more significant correlation between within-CCN connectivity and CDR-SOB, in which many of them were

**Figure 6-2:** Dynamic functional connectivity states results. The three identified dFNC states using the k-means clustering method. We found strong connectivity within-ADN, within-SMN, and within-VSN in all states. We found strong connectivity between SMN and VSN in state3. Also, this state showed negative connectivity between sensory networks, including ADN, SMN, and VSN, with the rest of the brain. We found that all subjects spend 23.78 %, 52.17 %, and 24.05 % in state 1, state 2, and state 3, respectively. The color bar shows the strength of the connectivity. SCN: Subcortical network, ADN: auditory network, SMN: sensorimotor network, VSN: visual network, CCN: cognitive control network, DMN: default-mode network, and CBN: cerebellar network.
positive. Also, within-DMN connectivity showed more negative connectivity compared with that of other states. This state also showed a positive correlation between connectivity between SMN and CBN, and CDR-SOB. Also, we observed both positive and negative correlations between CDR-SOB and between-network connectivity in state 2.

State 3 showed a significant and negative correlation between the within-SMN, within-VSN, within-DMN, within-CCN, and within-CBN connectivity and CDR-SOB. The amount of significant correlation between within-SMN, within-VSN, and between SMN and VSN connectivity with CDR-SOB was more than those of the other states. Also, this state showed a significant positive correlation between VSN and CBN connection and CDR-SOB. Overall, we observed a reorganized pattern in the correlation between CDR-SOB and between-network connection in this state.
6.3.3 The correlation between temporal properties of dFNC and CDR-SOB

We calculated the partial correlation between CDR-SOB and temporal features of dFNC (i.e., OCR and HMM) by controlling the age and gender. We found a positive correlation between OCR of state 1 and CDR-SOB ($r=0.07$, corrected $p=0.009$) and a negative correlation between OCR of state 3 and CDR-SOB ($r=-0.14$, corrected $p=2e-7$). Also, we observed a negative correlation between CDR-SOB and $a_{11}$, i.e., the transition from state 1 to state 1 ($r=0.07$, corrected $p=0.02$), and a positive correlation between CDR-SOB and $a_{33}$, i.e., the transition within state 3 ($r=-0.11$, corrected $p=0.0001$).

6.3.4 Both healthy and patient brains follow similar state pattern

Since the number of HC scans is more than the number of patient ones, we applied the clustering method to their dFNC of HC and patients separately. The results are shown in Figure 6-4. This figure shows that our approach captured a similar brain state in both groups. We used the Pearson correlation between states’ FNC to assess the similarity between them. The correlation between state 1 of HC with state 1 of the patient group, between state 2 of HC with state 2 of the patient group, and between state 3 of HC with state 3 of the patient group were $0.9903 (N=1378, p<0.001)$, $0.9825 (N=1378, p<0.001)$, and $0.9921 (N=1378, p<0.001)$, respectively (Figure 6-4A and Figure 6-4B). In addition, the OCR followed a similar pattern to the results when we concatenated all subjects. State 2 shows the highest OCR among all three states in both groups. HC subjects have higher OCR in state 1 than patients ($p<0.01$), while patients have higher OCR in state 3 than that HC subjects ($p<0.01$) (Figure 6-4C and Figure 6-4D). These results proved that the HC subject’s dFNC did not dominate the state pattern. In addition, to reference the states easier in this study, we put a name on each state. Since both healthy subjects and patients spend
more than 50% of their scanning time in state 2, we called this state a baseline. Since vmAD patients spend more time in state 1, we called this state the vmAD-related state, and finally, we call state 3, in which healthy subjects spend more in this state than state 2, an HC-related state.

6.3.5 Changing the number of states does not change the results

To test whether the number of clusters (or states) would change the results or not, we applied the same clustering method for $k=5$, $k=7$, and $k=10$. Figure 6-5A and Figure 6-5B

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Figure 6-4: Dynamic functional network connectivity in healthy control and patients. 
A) The three identified dFNC states using the k-means clustering method in healthy control (HC), B) Occupancy rate (OCR) of HC in each state. HCs have the highest OCR in state 2 (corrected $p<0.001$). OCR of state 3 is higher than the OCR of state 1 in HC subjects (corrected $p<0.01$), C) The three identified dFNC states using the k-means clustering method in patients (vmAD), D) Occupancy rate (OCR) of HC in each state. vmAD subjects have the highest OCR in state 2 (corrected $p<0.001$). OCR of state 1 is higher than OCR of state 2 in patients (corrected $p<0.01$). SCN: Subcortical network, ADN: auditory network, SMN: sensorimotor network, VSN: visual network, CCN: cognitive control network, DMN: default-mode network, and CBN: cerebellar network.
Figure 6-5: Evaluating the k value effect on the results. A) The dFNC states identified using the k-means clustering method with \(k=5\). B) The dFNC states identified using the k-means clustering method with \(k=7\). The colorbar represents the intensity of connectivity in each state. SCN: Subcortical network, ADN: auditory network, SMN: sensorimotor network, VSN: visual network, CCN: cognitive control network, DMN: default-mode network, and CBN: cerebellar network.
Figure 6-6: Evaluating the k value effect on the results. The dFNC states were identified using the k-means clustering method with k=10. The colorbar represents the intensity of connectivity in each state. SCN: Subcortical network, ADN: auditory network, SMN: sensorimotor network, VSN: visual network, CCN: cognitive control network, DMN: default-mode network, and CBN: cerebellar network.

, and Figure 6-6 show the results for k=5, k=7, and k=10, respectively. We observed similar state patterns with different k values. State 1 (k=3) is similar to state 1(k=5), state 2(k=7) and state 5(k=10). State 2 (k=3) is similar to state 2(k=5), state 1 (k=7), and state 8 (k=10). State3 (k=3) is similar to state 3(k=5), state 5 (k=7), and state7 (k=10). Increasing k above the optimized value of k=3 yields states whose similarity to those in the optimized value weakens as k grows. However, the two states (states 1 and 3) in the optimized clustering
whose OCR is significantly linked to CDR-SOB are highly replicable up to $k=7$ and whose occupancy has a replicable significant relationship to CDR-SOB. Table 6-2 shows the correlation between CDR-SOB with OCR of clustering with different $k$ values.

6.3.6 Dementia progression associated with functional network connectivity

Using baseline FNC features, including sFNC, HMM, and OCR, from whole-brain FNC, we successfully predicted the conversion from the normal state to vmAD by classifying those subjects converted to the mildly impaired stage (i.e., c-HC) from those who stayed unchanged within five years, i.e., uc-HC. The average accuracy, sensitivity, specificity, and AUC in this classification were 75%, 72%, 78%, and 81%, respectively.

6.4 Discussion

In this study, we explored the dynamic of whole-brain FNC of HC (CDR-SOB=0) and vmAD (0.5≤CDR-SOB≤9) subjects from the longitudinal rs-fMRI OASIS-3 [113]. Using a data-driven approach, we extracted 53 ICs for the whole brain and used a sliding window approach followed by a clustering method to study dFNC of this dataset. We found that the connectivity between VSN and SMN is dynamic by a transition from positive connectivity in state 3 to moderate positive connectivity in state 2 and negative connectivity in state 1. In addition, the connectivity between SMN and VSN with the rest of the brain changes from negative connectivity in state 3 to another state with more positive connectivity. Besides SMN and VSN, we found a dynamical pattern in CCN connectivity. Overall, we found that the whole-brain FNC is highly dynamic. This result argues the previous AD-related literature that mainly ignored the dynamic behavior of brain connectivity. Although a few studies explored the dFNC recently [125], [126], the current study uses Neuromark
as a replicable platform to extract data-driven ICs from relatively large longitudinal data. This replicable platform can generate independent components and replicate that across the different datasets. This would help reproduce similar brain states for FNC across different datasets, which is very important when studying dynamics [127].

We found that within-SMN FNC decreased by the transition from an HC to a vmAD. This pattern was observed in all three states. Previous studies showed a decrease in SMN FNC is shown in AD patients compared with that of HC subjects [128]–[131]. In more detail, we found that the FNC within the postcentral gyrus decreased in vmAD than in the HC brain. The postcentral gyrus has a key role in somatic sensation, including pain and temperature [132]. Also, a previous study showed an impairment of pain and temperature sensation in mild dementia [133]. Therefore, dysconnectivity in the postcentral gyrus can potentially explain the impairment of somatic sensation in the early stage of dementia and suggest a prospective study.

In addition, we observed that the VSN FNC, in the particular fusiform gyrus, decreases when the brain progress from a healthy state to mild dementia. As previous studies showed, the fusiform gyrus is involved in face recognition, and alteration in the connectivity

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Table 6-2: The link between the occupancy rate of each state and CDR-SOB
between this brain region and other subregions of VSN causes impairment in face recognition [134], [135]. Functional dysconnectivity between fusiform and the rest of the VSN potentially can explain the impairment of face recognition in the early stage of AD progression [136]. Also, results indicated reduced FNC among the brain sensory networks, i.e., ADN, SMN, and VSN, by progression from HC state to vmAD. Information processing integration of multisensory signals is a hallmark of self-awareness. For instance, [137] showed that the matching between visual perception and proprioceptive signals is necessary for preserving self-consciousness. Disconnection among sensory networks in mild dementia patients than that of healthy subjects can potentially explain the underlying mechanism of self-awareness discrepancy in AD patients. The current findings suggest future studies for exploring a causal link between dysconnectivity in the sensory network and lack of self-awareness in AD patients.

We also observed a disrupted temporal and spatial pattern in the connectivity between CBN and other brain networks. In all states, we found a decrease in the connectivity between CBN and SCN by advancement from the HC brain to vmAD. However, the connectivity between CBN and SMN and between CBN and VSN are higher in vmAD than in the HC subjects. This finding is consistent with a previous study that showed a reorganized pattern in the connectivity between cerebellar subregions and DMN, VSN, and SMN [116]. However, we did not detect a significant pattern in the correlation between the clinical rate and the connectivity between CBN and DMN.

In addition, we found a disrupted pattern in DMN connectivity by having reduced connectivity in state 3, and both increased and reduced connectivity in state 1 and state 2 for vmAD than the normal brain. Based on sFNC, previous studies reported both increase
and decrease in whole-DMN connectivity of the AD subject. Another study reported no significant difference in DMN connectivity between AD patients with HC. Although a small sample size might affect the statistical power, as previously shown in a study of major depression, this inconsistent result partially could be due to focusing on static FNC, which is obtained from the correlation within an entire time series. Similarly, we observed a disrupted spatial and temporal pattern in CCN connectivity. In addition, we found a reorganized pattern in the connectivity between CCN and other networks such as SCN, ADN, SMN, VSN, DMN, and CBN. A recent study showed a reorganized pattern in the connectivity between inferior parietal lobule, as a part of CCN, with default mode, salience, executive control, and sensorimotor networks. Our new finding provides new knowledge about the reorganized pattern between CNN and the rest of the brain. The decrease in the CCN FNC might explain the loss in the functional integrity of the CCN network, and the increased FNC showed that vmAD patients potentially utilize additional brain subregions to compensate for the impairment of cognitive function. We also found that the FNC within SCN, including caudate, thalamus, and putamen, decreased in vmAD patients compared with that of normal subjects. This result is consistent with a previous study that showed subjects with the risk of AD showed less connectivity in the caudate and thalamus.

Next, we calculated the correlation between clinical rate and OCR and HMM. We found HC subjects spend more time in state 3, which showed the highest positive connectivity among sensory networks, i.e., VSN, SMN, and ADN, is less in vmAD patients than the normal subjects. Also, we found that the dwell time of state1, which showed the least connectivity among sensory networks, is higher for the patients with mild dementia. This
finding provides further evidence of the effect of disease on the dysregulating temporal properties of FNC. A recent study showed that AD subjects spend more time in a sparsely connected state in which the motor network is isolated from the rest of the brain [125]. Our result is consistent with the study mentioned above by showing that the subjects with mild severity in the early stage of AD spend more time in state 1, which shows sparse connectivity among brain networks. However, another part of our result that shows normal brain spends more time in state 3, in which SMN is isolated from most parts of the brain except VSN, contradicts the result of the aforementioned study. In addition, spending more time in a state with lower connectivity between SMN and VSN and less time in a state with stronger connectivity between SMN and VSN by subjects in the early stage of AD emphasizes more on the role of this connectivity in the transition from the normal stage to the early stage of AD. Also, our result is consistent with another study from our group on a different dataset that showed that AD patients spend more time in a state with lower connectivity and spend less time in a state with higher connectivity [98].

Prior work has demonstrated the regional patterns of AD pathology and their overlap with DMN regions [144]. Therefore, we expected DMN to be impacted, as demonstrated in prior studies. However, we found that associations between primary sensory/motor networks were most correlative to symptom severity. Sensory and motor networks are considered relatively spared from AD pathology, at least until the later stages of the disease. These exciting findings suggest that although relatively preserved and potentially due to high signals in these regions, regions involved in cross-modal sensory/motor integration are damaged. This information provides a sensitive measure of neural damage in AD (potentially more sensitive than primary degeneration regions). Our result might
suggest that DMN is the last brain network that is affected by AD. Our result might also explain the previous study’s finding that motor function changes might predate cognitive impairments and dementia onset [145], [146]. However, a prospective study is needed to find which specific sensory or motor function changes sign early AD. Also, our new result about dysconnectivity in the somatosensory network might explain why physical exercise would prevent AD [147] by increasing FNC among sensory networks [148].

Neurofeedback is a form of real-time biofeedback regulating brain activity and promoting brain function and behavioral performance[149]. In this technique, the neural signals are recorded from the brain. A feedback mechanism is then used to control the neural signal features through a feedback loop in the form of audio, video, or a combination of them. This closed-loop therapy has been widely used for the major depressive disorder [150], attention deficit hyperactivity disorder [151], and autism [152] and has gotten attention for treating AD in recent years. A recent study used the amount of delta, theta, alpha, and beta activity from EEG signals as a control signal in neurofeedback to improve cognitive function in AD[153]. We introduced the sensory network's connectivity as a potential control marker in the neurofeedback in the current study. More specifically, our result suggests a possible benefit of administering the neurofeedback during the vmAD-related state and switching the brain state from vmAD-related to HC-related state. Although many technical limitations of real-time implanting neurofeedback system integrated with dFNC exist [154], [155]. Our results suggest a future benefit of dFNC states in neurofeedback in AD treatment.

Finally, we show that both dFNC and sFNC can be used to predict the conversion from healthy to vmAD based on their baseline recording. Previous literature proposed a few
models to predict conversion from MCI to AD [156]–[158]. For example, one study used 75 state MCI, i.e., who did not convert to AD, and 51 progressive MCI, i.e., who changed to AD within 3 years, modeled an SVM and could classify them with 79.37 % accuracy based on the brain connectivity features [159]. Another study used structural and genetic data for prediction from converted normal subjects to mild cognitive impairment from the unconverted normal subject within 5 years and could predict the conversion from normal to mild cognitive impairment with an AUC of 85% [160]. However, the model for the conversion from the normal brain to a mild impairment state based on their baseline recording has not been extensively reported. The current study shows a potential for FNC in predicting from healthy aging to mild impairment stage.

6.4.1 Limitations and future work

There are some limitations to this work. The choice of window size is an implicit assumption about the dynamic behavior in that a short window captures more rapid fluctuations, whereas a longer window does more smoothing. Future work can be accomplished to evaluate the range of dynamics more comprehensively [161]. In addition, we used SVM to compute the classification between individuals who converted to vmAD, and those who did not convert. Other more advanced methods like neural network-based classification can potentially increase the prediction accuracy. However, applying neural network-based classification is almost impossible due to the limited number of samples in the longitudinal data used in this classification.

6.4.2 Conclusion

In the work reported here, we extend this existing body of knowledge into the dynamic realm, investigating how time-varying properties of whole-brain FNC change by the
transition from healthy aging to vmAD. We found a state-specific reorganized pattern in the whole-brain FNC of vmAD patients. We observed a decreased connectivity among sensory networks, including SMN, VSN, and ADN, in a mild dementia state. This provides a piece of new knowledge about the sensory network dysconnectivity in the early stage of AD with mild symptom severity. This potentially marked that the sensory network is one of the brain networks that got affected more than the other brain network in the early stage of AD. In addition, we found a reorganized pattern, i.e., both increase and decrease in DMN and CCN connectivity. A similar changed pattern was observed in between-networks connectivity. We also found that mild dementia is linked to the temporal pattern on FNC by increasing the amount of time staying in a sparsely connected state with lower functional connectivity among sensory networks. These results emphasized that not only the transition from the normal state to mild dementia changes the connectivity strength, but also it dysregulates the temporal properties of FNC
CHAPTER 7. THE LINK BETWEEN STATIC AND DYNAMIC BRAIN FUNCTIONAL NETWORK CONNECTIVITY AND GENETIC RISK OF ALZHEIMER’S DISEASE

7.1 Introduction

Alzheimer’s disease (AD) is the most prevalent age-related dementia in individuals above 65 years of age [31]. While global biomedical research efforts for AD prevention have expanded, the number of individuals affected by AD is still growing significantly every year. Even though there is no effective AD therapy to date, some medications can slow down disease progression [108]. It has been hypothesized that AD progression affects brain functional connectivity beginning many years prior to disease onset [162], [163]. As such, knowing how AD risk alters brain connectivity in cognitively normal individuals might shed light on the mechanisms associated with AD development later in life.

While previous studies showed that environmental factors such as diet, living in rural versus urban areas, smoking, not exercising, and infections are risk factors for AD, genetic factors are believed to contribute 70% to AD risk [164], [165]. Apolipoprotein E polymorphic alleles are genetic factors linked to Alzheimer’s disease (AD). There are three common alleles, including ε2, ε3, and ε4, that can produce six genotypes such as ε2/ε2, ε2/ε3, ε2/ε4, ε3/ε3, ε3/ε4, and ε4/ε4 in which one gene is inherited from the father and the other is from the mother [166]. Individuals carrying the ε4 allele have the highest risk of AD and younger mean age at dementia onset compared to those carrying the ε3 and ε2 alleles, whereas individuals with the ε2 allele have the lowest risk and older mean age of dementia onset [167].
Previous studies explored the link between AD's genetic risk and static functional network connectivity or sFNC [168]–[171]. For example, a recent study found that individuals carrying ε4 have lower temporal default mode network (DMN) functional connectivity than those without ε4 [170]. Another study showed an increase in functional connectivity between the hippocampus and prefrontal/parietal/temporal cortex in healthy individuals carrying ε4 [172]. Despite extensive research on the effect of ε4 on rs-fMRI sFNC, the effects of other alleles (i.e., ε2 and ε3) on sFNC have not been explored. Additionally, previous literature that studied the APOE effect on FNC assumed that FNC is static over time and ignored its dynamics. However, recent studies have shown that FNC is highly dynamic during both task and resting conditions [4], [173], [174]. Therefore, we hypothesized here that studying the dynamics of whole-brain FNC might give insight into how the APOE could disrupt FNC in AD.

This chapter aimed to explore how dFNC and sFNC differ among individuals with different genetic risks for AD using a relatively large dataset (N>850). To model the dFNC of each participant, we utilized a sliding window approach followed by k-means clustering to estimate a set of connectivity states [4]. Next, we modeled the temporal changes by calculating the occupancy rate (OCR) of each state from the dFNC. Next, we explored the difference between cognitively normal participants with different AD risk levels via statistical analysis of the estimated OCR features and the number of the between-state transitions. In addition, we compared sFNC cell-wise differences between cognitively normal individuals differing in genetic risk of AD.

7.2 Materials and methods

7.2.1 Participants and dataset
Neuroimaging data of 894 cognitively normal brains (362 females) and their associated demographic information from the longitudinal Open Access Series of Imaging Studies (OASIS)-3 cohort was used in this study [175]. The participants' cognitive functionality at the time of scanning was evaluated by the clinical dementia rating scale (CDR) scores, and the CDR scores were equal to 0. The participants' age at scanning time ranged from 42.46 years to 95.39 years, with a mean of 70.13 years. We divided the data into three groups, including the low genetic risk of AD or LGR_AD (N=135, 63 females), consisting of all individuals with ε2 allele (i.e., ε2/ε2 and ε2/ε3), moderate genetic risk of AD or MGR_AD (N=558, 219 females), containing all individuals with ε3 allele (i.e., ε3/ε3), and high genetic risk of AD or HGR_AD (N=201, 80 females) consisting of all individuals with ε4 allele (i.e., ε3/ε4 and ε4/ε4). No significant age, sex, and mini-mental state examination differences were observed between any pair of groups (p>0.05).

7.2.2 Imaging acquisition protocol

The imaging protocol is described in 6.2.2.

7.2.3 Preprocessing

This section is described in 6.2.3.

7.2.4 Dynamic and static functional network connectivity estimation

This section is described in 6.2.4.

7.2.5 Dynamic functional network connectivity clustering

We separated the windowed FNCs into a set of clusters (or states) with k-means clustering. The optimal number of clusters or k was set to three based upon the elbow criterion, e.g., “the ratio of within-cluster to between cluster distance. Pearson correlation was used as a
distance metric, and 1000 iterations were used. The output of k-means clustering was three states for each group and a state vector for each individual. The state vector represents changes in whole-brain FNC over time. Next, we calculated the proportion of each participant's time in each state, called occupancy rate (OCR) hereafter. Having three states, we estimated three OCRs for each individual.

7.2.6 Statistical analysis

A two-sample t-test was used to compare the OCR (number of null hypotheses or $N=3$) of each pair of groups. Similarly, a two-sample t-test was used to compare the sFNC ($N=1387$) of each pair of groups. We adjusted all p values with Benjamini-Hochberg false discovery rate (FDR) correction in both OCR and sFNC analysis [73].

7.3 Results

7.3.1 Genetics risk associated with sFNC

The average sFNC of each group is shown in Figure 7-1A. Also, Figure 7-1B shows the cell-wise FNC differences between each pair of groups. We used a two-sample t-test to find the differences between groups. Significant group differences that passed the multiple comparison tests are shown with an asterisk (FDR corrected $q=0.05$). In this figure, the red and blue colors show the positive and negative differences, respectively. Figure 7-1B (left panel) shows the cell-wise difference between LGR_AD and MGR_AD (MGR_AD-LGR_AD). We did not observe a significant sFNC difference between LGR_AD and
MGR_AD groups after FDR correction. While the difference between LGR_AD and HDR_AD (or HGR_AD - LGR_AD) was significant in some networks, as shown in Figure 7-1B (middle panel). However, the pattern was widespread across the whole brain.

In contrast, the cell-wise FNC difference between MGR_AD and HDR_AD (or HGR_AD - LGR_AD) was more focused on VSN, as shown in Figure 7-1B (right panel). As shown in this figure, we found that individuals with a higher risk of AD have less VSN connectivity than those with an intermediate risk of AD. In comparison, the connectivity

Figure 7-1: Estimated sFNC for each group. A) Estimated sFNC for LGR-AD (left panel), MGR-AD (middle panel), and HGR-AD (right panel), B) The sFNC difference between each pair of groups. Significant group differences passing the multiple comparison threshold are marked by asterisks (false discovery rate (FDR) corrected, q = 0.05). The colorbar shows the intensity of sFNC values. SCN: subcortical network, ADN: auditory network, SMN: sensory motor network, VSN: visual sensory network, CCN: cognitive control network, DMN: default mode network, and CBN: cerebellar network. LGR-AD: Low genetic risk of AD, MGR-AD: Moderate genetic risk of AD, HGR-AD: High genetic risk of AD.
between VSN and CCN and between VNS and DMN was higher for individuals with higher AD risk.

### 7.3.2 Dynamic functional network connectivity states

Figure 7-2 shows three distinct dFNC states estimated by k-means clustering. State1 and state 2 show more positive connectivity within CCN, within CBN, within SMN, and within VSN compared with state3. State 2 offers the most positive connectivity among sensory domains (i.e., ADN, SMN, and VSN). Meanwhile, the connectivity between these three domains with the rest of the brain is negative in this state. Additionally, the connectivity between SMN and the rest of the brain is relatively high in state1.

### 7.3.3 Genetics risk associated with dFNC features.
We compared the OCR of each state across three groups of individuals. The results are shown in Figure 7-3A. As this figure shows, we found that the OCR of state 1 was significantly less for HGR_AD than that of LGR_AD. In contrast, HGR_AD had higher OCR than LGR_AD in state 3. Additionally, we did not find any significant OCR
difference across groups in state 2. Besides, no significant difference was observed between MGR_AD and LGR_AD and between HGR_AD and MGR_AD.

### 7.3.4 Gender effect on sFNC and dFNC features

To consider sex effects on the results, we separated men and women in each group of individuals and repeated our analysis. The group sFNC difference for each sex is shown in

**Figure 7-4: Sex effects on sFNC.**

- **A)** sFNC differences between pairs of groups for men.
- **B)** sFNC difference between pairs of groups for women. Significant group differences passing the multiple comparison threshold are marked by asterisks (false discovery rate (FDR) corrected, q = 0.05). The colorbar shows the intensity of sFNC values. SCN: subcortical network, ADN: auditory network, SMN: sensory motor network, VSN: visual sensory network, CCN: cognitive control network, DMN: default mode network, and CBN: cerebellar network. LGR-AD: Low genetic risk of AD, MGR-AD: Moderate genetic risk of AD, HGR-AD: High genetic risk of AD
Figure 7-4. Figure 7-4A shows the sFNC difference for each male participant group, and Figure 7-4B shows similar results for women. Interestingly, we did not find any significant sFNC difference across groups for males. In contrast, the sFNC difference between LGR_AD and HGR_AD was significant for the women, as shown in Figure 7-4B (left panel). We did not observe a significant difference between women LGR_AD and MGR_AD and between women MGR_AD and HGR_AD.

We also studied group differences in dFNC features for both men and women separately. We did not observe a significant group difference in the OCR of each state (Figure 7-3B) for men individuals like sFNC result. In comparison, the group difference was significant for the women (Figure 7-3C). In more detail, we found a significant OCR difference between LGR_AD and MGR_AD and between LGT_AD and HGR_AD in state 1 and state 3. At the same time, sFNC features did not differentiate between these groups.

7.4 Discussion

Previous studies showed that FNC estimated from rs-fMRI is highly dynamic even without external input [4], [173]. Therefore, here we hypothesized that the genetic risk of AD not only alters the strength of the functional connectivity between pairs of brain networks, as would be shown in sFNC, but also the dynamic fluctuations of connectivity among those networks, as would be shown in dFNC. To the best of our knowledge, the present study is the first to report a link between AD genetic risk and dFNC estimated from rs-fMRI recorded by cognitively normal participants. We also compared the results obtained from both sFNC and dFNC data as the measures are complementary and could provide distinct insights into the relationship between brain network connectivity and the genetic risk of
AD. Lastly, we examined the effects of an individual’s sex on the degree to which sFNC, dFNC, and genetic risk of AD are associated.

We used a large dataset and data-driven, reproducible methods in our study. We used 6-minute sessions of rs-fMRI data from 894 cognitively normal individuals with different AD genetic risks and put them into three groups, including individuals carrying at least one ε2 (i.e., ε2/ε2 and ε2/ε3), individuals carrying only ε3 (i.e., ε3/ε3), and individuals carrying at least one ε4 (i.e., ε3/ε4 and ε4/ε4). We used a sliding window approach to calculate the whole-brain dFNC over time and k-means clustering to partition the whole-brain dFNC into three distinct states. Next, we compared the OCR for each state among individuals with different genetic risks for AD. We also compared sFNC across individuals with different risks of AD.

We observed a widespread effect of genetic risk on the sFNC of cognitively normal individuals, which is consistent with previous studies [176], [177]. We found that genetic risk affects the VSN significantly more than other brain networks. In more detail, we found that individuals carrying ε4 have less within-VSN functional connectivity compared to those individuals carrying ε3 and not ε2. A decrease in VSN activity during a visual task has been reported in individuals carrying ε4 [178]. A recent study reported functional connectivity decreases in the primary, secondary, and associative visual cortices for the cognitively normal individuals with the ε4 allele compared with ε4 allele non-carriers [171]. We also found that cognitively normal individuals carrying ε4 have higher VSN/CCN than individuals with moderate AD risk and more increased VSN/DMN connectivity relative to individuals with moderate and lower AD risk. We hypothesize that this higher VSN/CCN connectivity in ε4 carriers could be a compensatory mechanism to
offset the VSN FNC reduction in these individuals. A similar compensatory mechanism in functional connectivity has been reported in individuals with ε4, enabling them to achieve the same performance level as individuals with ε3 in a memory task [179]. It is worth mentioning that we did not find a significant sFNC difference between the individuals with the lowest risk and the moderate risk of AD, which suggests that a modest risk of AD does not change the brain FNC to the extent that the highest risk of AD does.

We also explored the whole-brain dFNC across cognitively normal individuals with different genetic risks for AD. We observed a significant difference in the OCR of individuals carrying ε4 and individuals having ε2. At the same time, we did not observe any significant differences between those with ε2 and ε3 and those with ε3 and ε4. In more detail, we found that individuals carrying ε4 spend more time than those carrying ε2 in state3 with lower within-VSN functional connectivity (relative to state1 and state2). This result is consistent with the result obtained from the sFNC data, which showed that individuals with higher AD genetic risk have less within-VSN functional connectivity. That supported our hypothesis about the genetic risk effects on both the strength and the temporal pattern of FNC estimated from rs-fMRI.

We found that individuals with ε4 spend more time in state3 with lower SMN, lower CCN, and lower CBN functional connectivity (relative to state1 and state2), while we did not observe a significant difference in those networks by analyzing sFNC data. These results might reveal some new evidence of the CCN, VSN, and CBN in differentiating individuals with different genetic risks for AD. We did not observe a significant difference in OCRs of the individuals with a moderate and lower risk of AD, consistent with the sFNC analysis.
results. These evidence might suggest that a moderate genetic risk of AD does not affect either brain sFNC or dFNC.

We observed a considerable difference in sFNC when comparing the individuals having ε3 with those having ε4. However, no significant difference between the two groups was observed by looking at OCR. While previous studies only analyzed sFNC [168]–[171], the results reported above demonstrated the importance of examining both sFNC and dFNC data to differentiate individuals with different AD risks.

We also separated men and women to examine the effect of sex on our results. We did not observe a significant difference across these three groups of men for either sFNC or dFNC data. While a significant difference between women carrying ε4 and ε2 and between women carrying ε4 and ε3 was observed in the dFNC analysis, we only found a significant difference between the women with ε2 and ε4 in the sFNC analysis. We did not observe a significant difference between individuals having ε3 and ε4 in either analysis. A recent study analyzed 5496 healthy individuals carrying ε4 and showed that AD's conversion rate is significantly higher for women [180]. Our current research shows that sex differences significantly contribute to differentiating cognitively normal individuals with different AD genetic risks based on their rs-fMRI, which might explain the study above's finding. Although previous studies considered the ε3 allele the be a neutral factor in AD, a recent study claimed that the ε3 allele might be a protective factor rather than a neutral one [181]. Our results may suggest that the ε3 allele is a more significant risk factor for AD in women than in men.

7.4.1 Limitations and future work
It should be noted that our study does have some limitations. In particular, previous studies have indicated that risks in addition to genetic risk can lead to AD [182]–[184]. For example, a recent study showed that individuals with diabetes and the ε4 allele demonstrated a faster functional decline than those without diabetes [184]. Other confounding factors like smoking [183], physical activity [185], and education levels [186] could introduce some bias into our results. This information was not included in the dataset. Future studies are needed to explore AD genetic risk factors combined with other potential risk factors in both sFNC and dFNC data.

7.4.2 Conclusion

In conclusion, by analyzing the link between AD genetic risk with sFNC and dFNC for the first time, we found that AD genetic risk affects both sFNC and dFNC and that each analysis provides information about different aspects of the effects of AD risk on brain connectivity. When analyzing sFNC data, it was possible to differentiate people with lower risk from those with higher risk and people with moderate risk from those with higher risk. An analysis of dFNC showed that people with low risk could be discriminated from those with higher risk and that the SMN and CBN helped differentiate the two groups. When analyzing sFNC and dFNC from individuals of both sexes, we found that a higher risk of AD is associated with a reduction in within-VSN connectivity and an increase in VSN/DMN connectivity, potentially as a compensatory mechanism. However, when analyzing only women, we did not observe a similar compensatory mechanism. The lack of a compensatory mechanism in women could explain the higher AD conversion rate in women that has been identified in previous studies [180]. Additionally, our findings suggested that having only an ε3 allele could be a risk factor in women more than in men.
Our results shed new light on the genetic risk interactions for AD and brain connectivity in cognitively normal individuals and could assist future diagnostic and treatment efforts.
CHAPTER 8. ABERRANT DYNAMIC FUNCTIONAL CONNECTIVITY OF DEFAULT MODE NETWORK IN SCHIZOPHRENIA AND LINKS TO SYMPTOM SEVERITY

8.1 Introduction

In recent years, functional network connectivity (FNC) obtained from resting-state functional magnetic resonance imaging (rs-fMRI) time series has revealed a great deal of knowledge about brain dysconnectivity in schizophrenia[17], [80]. Among intrinsic brain networks, the default mode network (DMN) – includes the anterior cingulate cortex (ACC), posterior cingulate cortex (PCC), precuneus (PCu), medial prefrontal cortex (mPFC), ventral ACC, and lateral and inferior parietal cortex – has been widely studied due to its putative role in the underlying external monitoring, spontaneous cognition, and autobiographical thinking[187] and its links to multiple mental disorders including schizophrenia[188].

In DMN, the cingulate cortex, including posterior (PCC) and anterior (ACC), are involved in several complex cognitive functions, including decision-making, empathy, emotion, socially driven interactions, and autobiographical memory [189], [190]. Several studies showed a functional and structural alteration within- and between the cingulate cortex and other regions, which emphasized the role of this region in the pathology of schizophrenia [100], [191]–[199]. Using a voxelwise comparison between SZ and HC, SZ individuals show a reduction of ACC gray matter [200]. In addition, a reduction of ACC functional connectivity with regions associated with DMN has been associated with SZ [201]. Regarding PCC, a reduction of PCC gray matter volume has been shown in schizophrenia and their non-psychotic siblings[193]. One rs-fMRI study showed higher connectivity
between PCC and PCu in schizophrenia subjects [197]. Consistent with this, an increase in connectivity between PCu and PCC has been reported in schizophrenia subjects and their sibling [191]. In a small sample size, lower functional connectivity of ACC in anterior DMN and PCu in posterior DMN of schizophrenia subjects with schizophrenia subjects exhibiting poor insight is reported [202].

Several studies from our group and others have previously reported a link between the connectivity among ACC, PCC, and PCu and symptom severity in schizophrenia [203–205]. One of those studies reported a positive correlation between PCu/PCC connectivity and the symptom severity as measured by the scale for the assessment of positive symptoms (SAPS) in a relatively small number of subjects [205]. A study showed aberrant connectivity within DNM and that DMN connectivity correlates with symptom severity in schizophrenia subjects [206]. Another study found a link between the ACC thickness of SZ subjects and the duration of illness and severity of psychotic symptoms [200].

All the studies mentioned above either studied the DMN as a whole or emphasized the separate role of PCC, ACC, and PCu within DMN and their connectivity in the pathology of schizophrenia. However, inconsistent results in the functional connectivity among these regions have been observed frequently. For example, previous studies showed that SZ subjects had both increased and decreased ACC connectivity within DMN compared with HC [198], [207]. Although this inconsistency could, to a limited extent, be because of differences in disease subtypes or symptoms, we theorize that piece of the heterogeneity is driven by the emphasis on static functional network connectivity (sFNC) measure of functional connectivity obtained which represents an average across different brain states during an unconstrained resting state.
Unlike conventional sFNC, which is obtained from the correlation within an entire time series, dynamic functional network connectivity (dFNC) refers to the connectivity between any pair of brain regions (or networks) within sub-intervals of time series [117]. In fact, dFNC research suggests that cognitive deficits and clinical symptoms associated with many neurological disorders not only depend on the strength of the connectivity between any pair of brain regions but also on the variation of connectivity strength of those regions over time [19]–[21], [117], [208]–[211].

The temporal feature of dFNC has been reported as a plausible biomarker in finding the fundamental mechanism of the difference between healthy individuals and schizophrenia subjects [18]–[21]. A previous whole-brain dynamic connectivity analysis showed that schizophrenia subjects spend less time in a highly-connected state [19]. Another study from our group showed an abnormal pattern in the dFNC of DMN by comparing state-based connectivity strength, dwell time, and between-state transition number of HC and SZ subjects [21]. This study identified an SZ-associated pattern in the temporal dynamics of DMN in SZ subjects by showing that they spend more time in a state with sparsely connected nodes. Also, this study demonstrated a state-specific spatial disruption within DMN by showing that the central hubs of PCC and anterior medial prefrontal cortex are significantly impaired in SZ subjects. However, this study did not show how symptom severity is associated with this abnormal pattern. Also, in contrast with the mentioned study, which used a seed-based approach to extract the brain network components (regions), in the current study, we used a framework called NeuroMark [98]. NeuroMark is a fully automated independent component analysis (ICA) framework that uses spatially constrained ICA to estimate comparable features across subjects by taking advantage of
the replicated brain network templates extracted from two N~900 normative resting fMRI data sets. We analyzed the dFNC of data-driven DMN subnodes based on the NeuroMark template and showed an aberrant temporal pattern and a link between this connectivity pattern and symptom severity in schizophrenia.

To investigate the temporal dynamics of FNC within DMN subnodes connectivity, we used two different datasets. With a sliding window approach and later k-means clustering, the dFNC was applied to identify a set of connectivity states [117]. Further, to investigate and model the temporal changes in dFNC, we estimated the transition probability via a hidden Markov model (HMM) applied to the dFNC data. In the next step, via statistical analysis on the estimated HMM features, we tested for links between symptom severity and abnormal DMN dFNC in schizophrenia. Finally, to investigate within-state variability across all subjects, we utilized an interpretable machine learning approach, called elastic net regularization (ENR), to identify the most important feature in differentiating SZ from HC subjects [212]. This approach can model the difference between SZ and HC individuals in the connectivity of these subnodes of DMN in each state. We hypothesized the state-dependent connectivity disruption within a shorter timescale would reveal more information about the dynamics among DMN subnodes in schizophrenia and potentially can explain the heterogeneous results within these subnodes. Also, the investigation of the link between symptom severity and dFNC within these three network subnodes provides additional insight into the link between functional connectivity dynamics and clinical phenomenology.

8.2 Materials and methods

8.2.1 Participants and dataset
Data were obtained from the Mind Research Network Center of Biomedical Research Excellence (COBRE) [213] and the Functional Imaging Biomedical Informatics Research Network (FBIRN) [93] projects. The COBRE dataset includes 89 healthy controls (HC) and 68 schizophrenic subjects. The FBIRN dataset contains 151 SZ subjects and 160 HC. The raw imaging data were collected from seven sites, including the University of California, Irvine; the University of California, Los Angeles; the University of California, San Francisco; Duke University/the University of North Carolina at Chapel Hill; the University of New Mexico; the University of Iowa; and the University of Minnesota. In this study, the written informed consent was obtained from all participants. Institutional review boards approved the consent process of each study site. In the COBRE dataset, the subjects’ eyes were open during the scanning while the FBIRN subjects’ eyes were closed. Please see Supporting Information for a detailed description of the MRI acquisition system.

All SZ subjects were on a stable dose of antipsychotic medication, either typical, atypical, or a combination, for at least two months. The demographic information for these subjects is shown in Table 8-1 and Table 8-2. Using a two-sample t-test, we did not observe a significant difference between the age of HC and the SZ group in both datasets. A diagnosis of schizophrenia is confirmed with the SCID-IV interview [214], and an absence of schizophrenia diagnosis in HC is confirmed with the SCID-I/NP interview [215]. In addition, HCs with a first-degree relative with an Axis-I psychotic disorder diagnosis was also excluded. Symptom scores were determined based on the positive and negative syndrome scale (PANSS) [94].

8.2.2 Data processing
The same preprocessing as described in other chapters is used here. To extract reliable DMN independent components (ICs), we used the Neuromark automatic ICA pipeline within the group ICA of fMRI Toolbox (GIFT, http://trendscenter.org/software/gift), which uses previously derived component maps as priors for spatially constrained ICA [98]. The Neuromark automatic ICA pipeline was used to extract ICs by employing previously derived component maps as priors for spatially constrained ICA. In Neuromark, replicable components were identified by matching group-level spatial maps from two large-sample HC datasets. Components were identified as meaningful regions if they exhibited peak activations in the gray matter within DMN. Seven DMN subnodes were identified in DMN. This set of subnodes includes three subnodes in the PCu, two subnodes in the ACC, and two subnodes in the PCC. It is worth mentioning that with seven subnodes in DMN, we had twenty-one connectivity features, where each feature represents the strength of the connection between any pair of DMN subnodes (Step1 in Figure 8-1).

8.2.3 Dynamic functional network connectivity (dFNC)
Table 8-2 Demographic and clinical information of participants of FBIRN for each site

<table>
<thead>
<tr>
<th>Site 1</th>
<th>Number</th>
<th>Age</th>
<th>Gender (M/F)</th>
<th>PANSS (positive)</th>
<th>PANSS (negative)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>FBIRN</td>
<td>21</td>
<td>30.04±8.60</td>
<td>17/4</td>
<td>15.72±5.57</td>
<td>14.11±3.14</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>28</td>
<td>34.78±9.40</td>
<td>21/7</td>
<td>NA</td>
<td>NA</td>
<td>0.99</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>Site 2</th>
<th>Number</th>
<th>Age</th>
<th>Gender (M/F)</th>
<th>PANSS (positive)</th>
<th>PANSS (negative)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>FBIRN</td>
<td>12</td>
<td>44.91±11.34</td>
<td>12/0</td>
<td>16.90±6.70</td>
<td>17.20±7.39</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>38.10±9.39</td>
<td>7/3</td>
<td>NA</td>
<td>NA</td>
<td>0.62</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Site 3</th>
<th>Number</th>
<th>Age</th>
<th>Gender (M/F)</th>
<th>PANSS (positive)</th>
<th>PANSS (negative)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>FBIRN</td>
<td>24</td>
<td>44.41±11.90</td>
<td>19/5</td>
<td>16.95±4.43</td>
<td>16.87±5.91</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>27</td>
<td>42.48±12.56</td>
<td>21/6</td>
<td>NA</td>
<td>NA</td>
<td>0.99</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Site 4</th>
<th>Number</th>
<th>Age</th>
<th>Gender (M/F)</th>
<th>PANSS (positive)</th>
<th>PANSS (negative)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>FBIRN</td>
<td>26</td>
<td>36.88±12.82</td>
<td>21/5</td>
<td>14.29±3.77</td>
<td>13.41±4.64</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>26</td>
<td>35.23±10.56</td>
<td>20/6</td>
<td>NA</td>
<td>NA</td>
<td>0.99</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Site 5</th>
<th>Number</th>
<th>Age</th>
<th>Gender (M/F)</th>
<th>PANSS (positive)</th>
<th>PANSS (negative)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>FBIRN</td>
<td>14</td>
<td>36.64±10.27</td>
<td>9/5</td>
<td>13.14±4.46</td>
<td>15±6.28</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>37.53±9.76</td>
<td>10/5</td>
<td>NA</td>
<td>NA</td>
<td>0.99</td>
</tr>
</tbody>
</table>

<table>
<thead>
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<th>Site 6</th>
<th>Number</th>
<th>Age</th>
<th>Gender (M/F)</th>
<th>PANSS (positive)</th>
<th>PANSS (negative)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>FBIRN</td>
<td>29</td>
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<td>17/12</td>
<td>14.34±4.67</td>
<td>11.93±4.06</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>27</td>
<td>34.51±10.85</td>
<td>16/9</td>
<td>NA</td>
<td>NA</td>
<td>0.99</td>
</tr>
</tbody>
</table>

<table>
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<th>Site 7</th>
<th>Number</th>
<th>Age</th>
<th>Gender (M/F)</th>
<th>PANSS (positive)</th>
<th>PANSS (negative)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>FBIRN</td>
<td>25</td>
<td>39.56±11.80</td>
<td>20/5</td>
<td>16.12±5.41</td>
<td>14.04±6.82</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>27</td>
<td>37.56±10.75</td>
<td>20/7</td>
<td>NA</td>
<td>NA</td>
<td>0.99</td>
</tr>
</tbody>
</table>

Note: SZ: Schizophrenia; HC: healthy control; PANSS: Positive and Negative Syndrome Scale, M: Male, F: Female, NA: not applicable; all p values have been calculated using two-sample t-test.
For each subject $i = 1 \ldots N$, the dFNC of the seven subnodes in DMN was estimated via a sliding window approach, as shown in Step 2 in Figure 8-1. A tapered window was obtained by convolving a rectangle (window size = 20 TRs = 40 s) with a Gaussian ($\sigma = 3$) was used to localize the dataset at each time point. A covariance matrix was calculated to measure the dFNC (Figure 8-1 Step 1). The dFNC estimates of each window for each subject were concatenated to form a $(C \times C \times T)$ array (where $C=7$ denotes the number of ICNs, and $T=124$ in COBRE and $T=137$ in FBIRN denotes the number of windows), which represented the changes in brain connectivity between ICNs as a function of time [4], [117], [216]. Since the time resolution and the eye condition of these two datasets were different, we did not combine them in our study and analyzed them separately.

8.2.4 Clustering and latent transition feature estimation
A k-means algorithm was applied to the dFNC windows to partition the data into a set of separated clusters [4], [27], [117]. The optimal number of centroid states was estimated to be 5 using the elbow criterion based on the ratio of within to between cluster distance. The correlation was implemented as a distance metric in the clustering algorithm in 1000 iterations. Next, for each subject, we calculated the transition probability between states via HMM, and this probability was used as a latent feature of dFNC. The transition probability, $a_{ij}$, is the probability of the system transitioning from state $j$ at time $t$ to state $i$ at time $t+1$. (Step 3 in Figure 8-1). For each subject, twenty-five HMM features were obtained from five states.

8.2.5 **Quantifying group differences using the feature selection method**

Logistic regression (LR) classification was employed to quantify the difference between SZ subjects and HC based on the twenty-one connectivity features of each state. The process is shown in Figure 8-2. In this process, the FNC matrix of each window was converted to a vector. For the seven regions in the DMN, we obtained a total of twenty-one features (i.e., $C_1$, $C_2$, ..., $C_{21}$). Elastic net regularization, a machine-learning-based feature selection method, was used to model the difference between HC and SZ subjects [212], [217]. A machine-learning-based feature selection, which is not an assumption-dependent method, gives a generalized model of the difference between HC and SZ based on their connectivity features [103]. The LR model was fit using 10-fold nested cross-validation (CV) with a train-test ratio of 9:1 [218]. In the nested CV, an outer fold divided the data into training and test sets, while an inner fold divided the training data into another training and validation set. The optimized parameters are obtained using the inner-loop training and validation data. Here, the hyperparameters of each model are tuned to
minimize the inner-fold CV error of the generalization performance by sweeping the penalty parameter logarithmically from $10^{-5}$ to $10^5$. In addition, we computed the receiver operating characteristic (ROC) of the cross-validation was computed, and the area under the curve (AUC) as a measure of separability between SZ and HC. Finding the most informative feature in the classification between SZ and HC, during the sweep of the parameter in the inner fold, we calculated the proportion of models for which a given parameter was retained. This measurement may be interpreted as the relative importance of each feature in the classification. To identify the most important feature in ENR, we used multiple comparisons on a one-way analysis of variance (ANOVA) test and found those features equally had the highest contribution in the model classifying between HC and SZ subjects.

We then utilized elastic net regularization (ENR) as a feature selection approach to identify features that are most important to the classification of SZ vs. HC based on these twenty-one connectivity features. We chose to use ENR rather than the least absolute shrinkage and selection operator (LASSO) with L1-penalization since ENR employs both L1 and L2 penalization [212], [217] while LASSO depends only on L1. Elastic net is a regularization and feature selection technique that estimates the model parameters of the LR and selects the most important features by minimizing a cost function. This method applies both L1- and L2-regularization, as shown in Equations 8.1 and 8.2. In this method, the LR model parameters (i.e., feature coefficients) would be driven toward zero while $\lambda$ increases. This would give a trajectory of the model parameters as a function of $\lambda$ and form a regularization path of the model. The features corresponding to the slowest decaying coefficients were
interpreted as the most important ones. The cost function used in ENR is shown in the equations below:

$$\min_{\beta_0, \beta} \left( \frac{1}{2N} \sum_{i=1}^{N} (y_i - \beta_0 - x_i^T \beta)^2 + \lambda P_a(\beta) \right) \quad 8.1$$

$$P_a(\beta) = \frac{(1-\alpha)}{2} \|\beta\|_2^2 + \alpha |\beta|_1 \quad 8.2$$

where $N$ is the number of samples, $y_i$ is the label of sample $i$, $x_i$ is the feature vector of sample $i$, $\beta$ and $\beta_0$ are model parameters, $\lambda$ is the regularization parameters, and $P_a(\beta)$ is the penalty term in which $\alpha$, is a scaler value, determines the contribution of $L1$ or $L2$

**Figure 8-2: Feature selection.** The connectivity features of seven default mode network (DMN) subnodes were used as input to fit logistic regression as a classifier to discriminate SZ from HC. With seven subnodes of DMN, we had twenty-one connectivity features. Elastic net regularization (ENR), as a feature selection, used the model generated by the classifier and the input features to find the feature that was the most predictive in discriminating between the two classes. ACC: Anterior cingulate cortex, PCC: posterior cingulate cortex, PCu: Precuneus.
norms, in which $\alpha=1$ keeps only the $L_1$ and $\alpha=0$ keep only the $L_2$ norm [217]. In this study, the $\alpha$ was 0.95.

8.2.6 Statistical analysis

To find a link between twenty-five HMM features and PANSS of the SZ group, we used partial correlation by accounting for age, gender, and scanning site (for FBIRN). In addition, since the distribution of the HMM feature was not normal, we used the Spearman correlation method. All $p$ values have been adjusted by the Benjamini-Hochberg correction method for multiple comparisons [73]. Also, we used the $z$ score to test the effect size between two datasets [219].

8.3 Results

8.3.1 Dynamic functional connectivity states

Figure 8-3: Dynamic connectivity states results. A) The five identified dFNC states using the k-means clustering method in COBRE data, B) The five identified dFNC states using the k-means clustering method in FBIRN data. The similar states between the two datasets are aligned vertically. The similarity between states was measured by Pearson correlation of the cluster centroid matrix of two datasets. There is not a similar pattern between COBRE and FBIRN in state 5. The colorbar shows the strength of the connectivity.
Five states were identified in both datasets, as shown in Figure 8-3. For the simplicity of comparison, we aligned the similar states from both datasets vertically. The Pearson correlation between the cluster centroid matrix was used to quantify the similarity. Also, the state centroid values are shown in Table 8-3. A similar dynamic of DMN FNC is observed in both datasets, even though the eye condition was different. ACC regions showed negative connectivity in all states except state 5 in FBIRN data. The connectivity between ACC and PCC (ACC/PCC) is negative in all states. And the connectivity between PCu and PCC is positive in all states except state 3 of the FBIRN dataset. But within PCu, and within PCC, and between PCu and ACC showed both positive and negative connectivity.

### Table 8-3 Mean value of the connectivity in each state based on the cluster centroid matrix from Figure 8-3

<table>
<thead>
<tr>
<th>State</th>
<th>PCu</th>
<th>ACC</th>
<th>PCC</th>
<th>PCu/ACC</th>
<th>PCu/PCC</th>
<th>ACC/PCC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>COBRE</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>State1</td>
<td>-0.053</td>
<td>-0.142</td>
<td>0.145</td>
<td>-0.018</td>
<td>0.085</td>
<td>-0.060</td>
</tr>
<tr>
<td>State2</td>
<td>-0.004</td>
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<td>-0.050</td>
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</table>

**Note:** PCu: Precuneus, ACC: Anterior cingulate cortex, PCC: Posterior cingulate cortex, PCu/ACC: Connectivity between PCu and ACC, PCu/PCC: Connectivity between PCu and PCC, ACC/PCC: Connectivity between ACC and PCC.

8.3.2 *Difference between SZ and HC connectivity in each state*
Figure 8-4: Feature selection results in COBRE dataset. The left panel shows the ROC of the classification between SZ and HC in each state. The area under ROC or AUC of the SZ vs. HC classification was significantly higher than the change in all states. The right panel shows the most important features based on elastic net regularization that had equal and significant contributions to the classification. The colorful features are selected by multiple comparison ANOVA tests. AUC: Area under the curve.
Figure 8-5: Feature selection results in FBIRN dataset. The left panel shows the ROC of the classification between SZ and HC in each state. The area under ROC or AUC of the SZ vs. HC classification was significantly higher than the change in all states. The right panel shows the most important features based on elastic net regularization that had an equal and significant contribution to the classification. The colorful features are selected by multiple comparison ANOVA tests. AUC: Area under the curve.
A feature learning method embedded in a 10-fold LR classifier was used to identify the difference between SZ and HC subjects in each state. Figure 8-4 and Figure 8-5 showed the classification and feature learning results of each state in the classification between SZ and HC subjects in the COBRE and FBIRN datasets, respectively. Figure 8-6 shows the group difference between SZ and HC on the features selected by ENR selected in both datasets (corrected $p<0.05$). Red lines show stronger connectivity in HC compared to SZ subjects, and blue lines show stronger connectivity in SZ subjects compared to HC. Also, wider lines indicate stronger connectivity.

A disrupted PCu and PCC connectivity were observed in both datasets. In the COBRE dataset, SZ subjects showed a higher PCu and PCC connectivity in state 1, state 3, and state 4 and lower connectivity in state 2 and state 5 (corrected $p<0.05$). In FBIRN, PCu and PCC connectivity of SZ was higher in state 1, state 4, and state 5 (corrected $p<0.05$) and lower in state 3 (corrected $p<0.05$). Overall, the connectivity between PCu and the cingulate cortex (including both ACC and PC) of SZ subjects was higher in state 1, state 3, state 4, and state 5 for both datasets (corrected $p<0.05$). All states of FBIRN data showed a higher ACC connectivity in HC subjects, while in the COBRE data, only state 2 showed higher connectivity in HC subjects (corrected $p<0.05$), and there was not a significant difference between HC and SZ ACC connectivity in other states of this dataset. A higher HC connectivity between PCC and ACC was observed in state 1, state 2, state 4, and state 5 of COBRE data (corrected $p<0.05$). In addition, HC showed stronger connectivity between ACC and PCC in states 2 and 4 of FBIRN data (corrected $p<0.05$).

8.3.3 Symptom correlation with HMM features
It is important to understand how dynamic aspects of DMN connectivity correlate with symptom severity. In the COBRE dataset, we found one significant correlation between $a_{45}$ and positive PANSS (uncorrected $p < 0.05$). However, this correlation did not survive the false discovery rate (FDR) correction for multiple correlations. For the FBIRN data, only the correlation between $a_{25}$ and positive PNASS remained significant after multiple comparisons ($\text{Spearman } r = 0.21, N=144, \text{corrected } p=0.02$). Based on this result, the transition from state 5 with positive ACC and lower PCC/PCu connectivity to state 2 with negative ACC and relatively higher PCC/PCu connectivity significantly increased with increasing positive symptom severity. The effect size of the correlation of $a_{25}$ and positive PNASS is not significantly different between the two datasets ($|z|=1.5, p=0.07$). That means increasing the sample size of the COBRE dataset will possibly lead to a significant correlation result.

Figure 8-6: Group difference between SZ and HC connectivity in each state. Group differences in dFNC of those connectivity feature selected by the elastic net regularization method in each state (corrected $p < 0.05$). The wider line means a larger group difference. Red lines represent increased connectivity, while blue lines represent decreased connectivity in HC subjects (right panels). A) The group difference of twenty-one DMN connectivity features in the COBRE dataset, B) The group difference of twenty-one DMN connectivity features in the FBIRN dataset. ACC: Anterior cingulate cortex, PCC: posterior cingulate cortex, PCu: Precuneus.
8.4 Discussion

In the current study, we explored the temporal dynamics of functional connectivity among several data-driven DMN subnodes, including PCC, ACC, and PCu, and then the differences of connectivity features among these subnodes between SZ and HC subjects using rs-fMRI of two cohort schizophrenia datasets. In both datasets, we observed negative connectivity within ACC connectivity (except state 5 in FBIRN) and between ACC and PCC in all states. While the connectivity between PCu and PCC was positive in all states except state 3 in FBIRN dataset. On the other hand, the connectivity between PCu and ACC, within PCu, and within PCC connectivity showed a similar pattern in both datasets and fluctuated between positive and negative connectivity. Here, using data-driven subnodes within DMN, we proved that this brain network is highly dynamic. However, previous literature mainly ignored this dynamical behavior of DMN. In oppose to the previous study, which evaluated DMN dynamics using pre-defined regions of interest [188], the work presented here is the first study that utilized data-driven subnodes and compared the within DMN connectivity between SZ and H subjects and linked the temporal pattern of DMN with symptom severity in SZ subjects. As recent work has emphasized, it is essential to ensure the data within the node is consistent; otherwise, the results can be degraded or misleading [220]. This is especially true when we study dynamics [127].

The current study also extends the previous studies in which the dynamic of the whole-brain network of the same datasets was investigated [19], [40]. In a larger brain network, a group of brain networks such as the visual network, sensorimotor network, and auditory network, which are strongly correlated, may mask less-correlated networks and limit
spatiotemporal resolution [41]. That potentially can delineate why the main result of these studies was focused on these dominant networks and less reported about the dominated network such as DMN.

Previously, a few studies directly examined the functional connectivity of ACC in the pathophysiology of schizophrenia. However, inconsistent results in the ACC connectivity were observed. A study reported a lower ACC connectivity in SZ (number of subject or \( N=58 \)) subjects relative to HC (\( N=61 \)) ones [207]. A recent study showed a higher ACC connectivity for SZ (\( N=32 \)) subjects at the baseline to the HC (\( N=32 \)) and a decreased ACC connectivity after one week of olanzapine treatment [201]. In the current study, we found a disrupted pattern in the ACC connectivity in the smaller dataset (i.e., COBRE) in which only one state showed a higher ACC connectivity in HC subject, and no significant difference was observed in the other states. On the other hand, in the FBIRN dataset, which is a relatively larger dataset compared with COBRE and with those in the studies mentioned above, we found a consistently increased ACC connectivity of HC subjects in all states. One possible explanation for the previous inconsistent results is the small sample size. However, even in the smaller dataset, we highlighted an increased ACC connectivity in HC subjects by applying the dFNC approach. Therefore, studying the sFNC obtained from an unconstrained rs-fMRI could be another explanation for the inconsistent results in the connectivity of this region. Finally, a previous study in a relatively small number of subjects (\( N=13 \)) reported marginally (\( p=0.05 \)) greater within-PCC connectivity in SZ than HC [205]. However, in the current study, in both datasets with relatively larger sample size, no significant difference within PCC was observed in any state. This emphasizes the
importance of using data-driven subnodes to study within-PCC connectivity in schizophrenia pathophysiology.

Although most previous studies of DMN functional connectivity focused on ACC and PCC, we further highlighted the role of PCu/PCC connectivity by a comparison between HC and SZ subjects in two different datasets. In three states out of five ones, we found that the PCu/PCC connectivity is greater in SZ than HC subjects. However, we observed inconsistent results in different time spans. Using sFNC, previous studies reported both increase [191], [197] and a decrease[221] in the connectivity between PCu and PCC in schizophrenia. These contradicting results are possibly due to focusing on sFNC and averaging of the functional connectivity across time. The current study, which showed a disrupted pattern of PCu/PCC connectivity with a relatively large sample, potentially highlighted the importance of the study of functional connectivity in a shorter period.

Previous studies documented a higher activity in the ACC in any behavior relayed to decision-making[222]. Decision-making occurs at multiple timescales, which can be even in the order of hundreds of ms to several minutes[223]. This evidence, along with our result, showing that ACC connectivity is lower in SZ subjects in a short timescale may point to an underlying mechanism of decision-making deficit in schizophrenia. That might explain why we need to study the DMN FNC in a shorter span and further suggests the future investigation of the ACC connectivity in the decision-making behavior of schizophrenia subjects.

Also, we investigated the link between symptom severity and temporal pattern of dFNC in each subject. We found a significant positive correlation between symptom severity and the transition from a state with low PCu/PCC and high ACC connectivity to a state with
higher PCu/PCC and lower ACC connectivity in the FBIRN dataset. These results further emphasize the role of ACC connectivity and PCu/PCC connectivity as a potential biomarker of SZ, and this role is highlighted in the more severe SZ subjects. Our results suggest a possible benefit of changing the brain state with lower ACC connectivity and higher PCu/PCC connectivity and changing that state to a state with higher ACC and lower PCu/PCC connectivity (Figure 8-7).

A previous study explored the link between the whole-brain dFNC features, such as the number of transitions between state and dwell time of each state and the result was not significant after FDR correction [224]. Our current study showed that HMM features extracted from dFNC as a potential biomarker can link with symptom severity and support the importance of exploiting the dynamic information. This also motivated additional work to study the relationship of symptom severity to other dFNC features. In addition, based on the effect size analysis, we found that a larger sample of data is needed for a significant result. That might explain why the authors in the other study did not find a significant correlation between FNC dynamic and symptom severity [224].

**Figure 8-7: Potential clinical benefit of the result.** Our results suggest a possible benefit of changing the brain state with lower ACC connectivity and higher PCu/PCC connectivity and changing that state to a state with higher ACC and lower PCu/PCC connectivity.
Finally, as mentioned earlier, the eyes condition is different in the COBRE and FBIRN datasets. A previous study reported that different eye conditions might modulate the DMN dynamic [225]. That might explain some differences in the DMN dynamic between these two datasets. Among all states, state 5 of FBIRN dataset was detached from all states by showing higher within-ACC connectivity. Since previous literature showed higher activity in ACC during sleep [226], we wonder if this connectivity pattern possibly links to light sleep or drowsiness during the unconstrained state of eyes-closed condition in FBIRN dataset. That potentially proved another benefit for dynamic functional connectivity analysis, which can separate undesired states from the rest, specifically when the eye is closed.

8.4.1 Limitations and future work

There are some limitations to this work. Symptom scores are highly dependent on the skill and knowledge of the rater and the inclination of the subjects to be accurate in describing their symptoms[227]. The choice of window size is an implicit assumption about the dynamic behavior in that a short window captures more rapid fluctuations, whereas a longer window does more smoothing. Future work can be done to evaluate the range of dynamics more comprehensively [161]. Besides, while we are encouraged by the similarity of results across multiple data sets, schizophrenia is likely a heterogeneous disorder. More work is needed to evaluate the potential of multiple types of connectivity patterns within this group to provide additional insight into the disorder.

8.4.2 Conclusion

Previous studies focused on static connectivity of the DMN, including the PCC, ACC, and PCu, and showed an essential role of this connectivity in schizophrenia. In the current
work, we extended this existing body of research into the domain of dynamics by investigating the temporal patterns of connectivity in the DMN. A comparison of the DMN connectivity in SZs and HCs identified patterns of disruption in a shorter timescale that were reproducible across two relatively large datasets with distinct collection protocols. These patterns of disruption could possibly explain why previous studies of DMN connectivity showed contradictory results. In both datasets, we found that SZ subjects with higher symptom severity are more likely to transition from a state with lower PCu/PCC connectivity and higher ACC connectivity to a state with higher PCu/PCC connectivity and lower ACC connectivity. This highlights the potential relationship between symptom severity and the dysregulation of the dynamical properties of DMN functional connectivity.
CHAPTER 9. MULTIPLE OVERLAPPING DYNAMIC PATTERNS OF THE VISUAL SENSORY NETWORK IN SCHIZOPHRENIA

9.1 Introduction

Schizophrenia is a common, chronic, developmental brain disorder thought to involve aberrant connectivity [228]. In recent years, functional connectivity data derived from resting-state functional magnetic resonance imaging (fMRI) time series has proven highly informative regarding underlying brain connectivity patterns in schizophrenia [229], [230]. The disease has been characterized as one of “brain dysconnectivity” [231]. More recently, work has focused on the dynamics of functional network connectivity or dFNC [15], [19], [232]–[235]. Visual processing impairment is a known problem in SZ, and the visual sensory network (VSN) likely plays a key role in the disorder. However, there has not to date been a focused study on the resting fMRI dynamics among visual-related regions in schizophrenia.

Sensory processing dysfunction, including visual sensory, was reported in schizophrenia [236], as was an impairment of perceptual closure and its positive correlation with symptom severity [237]. The latter study also reported a reduction in visual cortex intrinsic connectivity. A reduction in the P1 component of the visual event-related potential highlighted an early visual processing deficit in schizophrenia subjects and their relatives [238], [239], suggesting that this may be an endophenotype.

All of the above studies show the potential role of the visual processing regions as an underlying mechanism in schizophrenia. Also, dFNC is informative in studying the
underlying mechanisms of brain function in many diseases [15], [19], [27], [119], [232]–[234]. Thus, we hypothesized that studying the dynamics of the visual network may be informative about in what manner and the degree to which the visual system is disrupted in schizophrenia. We expect that the state-dependent connectivity disruption within a shorter timescale would reveal more information about the dynamics of VSN in schizophrenia, and potentially the investigation of the link between visual learning and VSN dFNC provides additional insight into visual learning impairment in SZ subjects.

In this study, we leveraged the sliding window approach followed by $k$-means clustering to identify a set of connectivity states to investigate dFNC within the visual network [4]. To further investigate and model these temporal changes, we estimated the occupancy rate (OCR) of each state from dFNC. Next, via statistical analysis on the estimated OCR features, we explored the difference between schizophrenia and healthy subjects. In addition, we compared cell-wise differences between SZ and HC within each state. Finally, we investigated the link between OCR features and visual learning in SZ subjects.

9.2 Materials and methods

9.2.1 Participants

We used rs-fMRI and clinical data from the Functional Imaging Biomedical Informatics Research Network (FBIRN) projects [240]. The detail of clinical, demographic, and imaging protocol has been described in Chapter 8.

9.2.2 Visual learning

All participants had normal eyesight and were fluent in English. The visual figure learning test (VFLT) from the computerized multiphasic interactive neurocognitive diagnostics system (CMINDS) was used to test the visual learning in the participants [240], [241] and
was performed outside of the scanner. Six geometric figures in a 3×2 matrix were presented to participants for 10 seconds. Then, subjects are asked to draw as many of the presented figures as they can recall on a blank screen in the same location as they were presented originally. Around 25 minutes after completing the immediate recalling test, the subjects are asked to reproduce as many of the 6 original figures as they can recall. Next, 12 figures, including 6 original and 6 new, were presented to the subjects, and they were asked to answer “No” if the figure was new and “Yes” if the figure was old. The subject learning memory is calculated based on both accuracy and location and z-scored across all subjects. The details of this cognitive task have previously been published [240]. Table 9-1 shows the demographic, clinical, and visual learning task results for each site.

9.2.3 Data processing

The preprocessing has been described in the previous chapters. To extract reliable VSN independent components (ICs), we used the Neuromark automatic group ICA pipeline within GIFT (http://trendscenter.org/software/gift), which uses previously derived components maps as priors for spatially constrained ICA [98]. The Neuromark automatic group ICA pipeline was used to extract reliable, independent components (ICs) by employing previously derived component maps as priors for spatially constrained ICA. In Neuromark, replicable components were identified by matching group-level spatial maps
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<th>Antipsychotic medication</th>
<th>Illness duration (years)</th>
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**Total** | 151 | 38.06±11.30 | 115/36 | -1.09±1.15 | 303.26±160.01 (N=119) | 18.03±1245 |
from two large-sample HC datasets. Components were identified as meaningful regions if they exhibited peak activations in the gray matter within VSN. Although, as a previous study showed that group ICA is robust to the motion artifact [242]. Nine components were identified in VSN are shown in Step 1 of Figure 9-1.

9.2.4 Dynamic functional network connectivity

To calculate dFNC, we applied a sliding window approach, as shown in Figure 9-1. We used a tapered window obtained by convolving a rectangle (window size = 20 TRs = 40 s) with a Gaussian ($\sigma = 3$) to localize the dataset at each time point. Pearson correlations were calculated to measure the dFNC between 9 subnodes in VSN. With 9 subnodes in VSN, we estimated 36 identical connectivity features. The dFNC estimates of each window for
each subject were concatenated to form a \((C \times C \times T)\) array (where \(C=9\) denotes the number of ICs, and \(T=137\)), which represented the changes in brain connectivity between ICs as a function of time (Step 2 of Figure 9-1) [117].

9.2.5 \textit{K-means clustering and dynamic modeling}

A k-means algorithm was applied to the dFNC windows to partition the data into a set of separated clusters [4], [27], [117]. The optimal number of the centroid cluster was estimated based on the elbow criterion, which is the most common method for finding the optimal value of k in k-means clustering [19]. It is defined as the ratio of within-cluster to between cluster distance, and the objective function is to minimize this ratio. Through this procedure, we found that 5 was the optimal number by searching from \(k=2\) to 8. The correlation was implemented as a distance metric in the clustering algorithm in 1000 iterations. The output of k-means clustering includes 5 distinct states for the group and a state vector for each individual. A state vector shows how the network changes between any pair of states over time. In the next step, using the state vector, we found the portion of time each subject spent in each state. We called this feature the OCR of each state (Step 3 of Figure 9-1). With 5 states, we had 5 OCRs for each subject.

9.2.6 \textit{Statistical analysis}

We compared the functional connectivity feature of each state and OCR features between SZ subjects and HC subjects using two-sample t-tests. The connectivity feature comparison was made on 36 features, while the dFNC comparison was carried out on five OCRs features. In addition, to find the link between OCR features and the visual learning score, we used partial correlation accounting for age, gender, illness duration, antipsychotic
medication doses, and scanning site. In all statistics, $p$ values were adjusted by the Benjamini-Hochberg correction method for multiple comparisons.

9.3 Results

The following subsections highlight the main results of analyzing dFNC of VNS in SZ and HC subjects in this study. These include an overview of clinical measurement, dFNC states, the regional connectivity difference between HC and SZ in each state, and the difference between HC and SZ in OCR and its correlation with visual learning.

9.3.1 Demographic and clinical characteristics

The demographic and clinical characteristics of the participants is shown in Table 9-1. In SZ group, the age mean and standard deviation are 38.06 and 11.30, respectively. For HC one, the age mean and standard deviation of age were 37.04 and 10.68, respectively. By using a two-sample t-test on all subjects (combining all sites), we did not find a significant group difference between HC and SZ in age and gender. By combining all subjects from seven sites, SZ subjects showed significant impairment in visual learning when compared to HC subjects based on the two-sample t-test ($t(309)=9.22, p=1.18e^{-10}$). This difference was not significant in site 2, possibly due to their small sample sizes (see Table 9-1).

9.3.2 Overview of dynamic FNC states

By applying k-means clustering to the dFNC across all subjects, including HC and SZ, we found five distinct clusters (states). These five reoccurring dFNC states are shown in Figure 9-2. In state 1 and state 2 the middle temporal gyrus had a negative correlation with other regions of the VSN, while other regions showed a positive correlation with each other. In state 2, we observed stronger connectivity between the lingual gyrus, cuneus, and calcarine gyrus than in state 1. In state 3, the middle occipital gyrus had a strong negative
connection with other regions. Compared with other states, state 4 showed more positive connectivity between the inferior occipital gyrus and middle temporal gyrus. Another main characteristic of state 4 was weaker connectivity between the calcarine gyrus and other regions except for the cuneus and lingual gyrus. In addition, in this state, the connectivity within the middle temporal gyrus network was stronger than that of other states. In state 5, FG showed negative connectivity between FG and other regions.

9.3.3 Regional connectivity differences between SZ and HC within each state

To find differences between HC subjects and SZ subjects in each state, we used two sample t-tests. The results are shown in Figure 9-3. Significant group differences passing the multiple comparison testing are marked by asterisks (false discovery rate [FDR] corrected,
Interestingly, all states showed significant differences between SZ subjects and controls. In addition, we saw overlap in many of the cells showing HC vs. SZ differences. This suggests dynamics within the same sets of regions show unique fluctuations that differ significantly between HC and SZ subjects. State 3 showed the least overlap with other states. Interestingly, cuneus connectivity was significantly higher in HC subjects than SZ subjects in all states of dFNC. A similar pattern was observed in the connectivity with the middle temporal gyrus. States 2, and 3 showed fewer differences between HC and SZ in the connectivity of calcarine gyrus with other regions of VSN. The most differences between the calcarine gyrus and other regions were observed in state 4. In state 5, we observed the greatest difference between HC and SZ in the connectivity of the middle...
temporal gyrus and other regions within the VSN. Only state 5 showed a significant difference in the connectivity of lingual and fusiform gyri between HC and SZ. It worth mentioning that we investigated the effect of illness duration and antipsychotic medication dose on the state-specific functional connectivity, and we did not observe a significant effect of illness duration and medication dose on that of the SZ subjects.

Figure 9-4: Occupancy rate difference between HC and SZ and link with visual learning. A) The difference between SZ and HC occupancy rate in different states. The occupancy rate of SZ subjects is significantly higher than that of HC subjects in state 1 (FDR corrected $p=0.001$). In this graph, the mean value is shown by the black horizontal line in each violin, B) the correlation between visual learning score and OCR of state 4 in SZ subjects ($r=0.24$, corrected $p=0.04$. $N=119$). In this correlation, we accounted for age, gender, Antipsychotic medication, illness duration, and scanning site.
9.3.4 Occupancy rate differences between HC and SZ and links with visual learning

Figure 9-4A shows the OCR distribution of SZ and HC in each state. Using a two-sample t-test, we found that the OCR of state 1 was lower in HC than that in SZ (FDR corrected $p=0.001$). There were no significant differences in OCR between SZ and HC in any of the other states. In addition, we used a partial correlation between the visual learning score of SZ subjects and their OCR of different states while controlling for age, gender, and scanning site. The correlation between state 4 OCR showed a positive and significant correlation with visual learning memory in SZ subjects ($r=0.24$, corrected $p=0.04$, $N=119$), as shown in Figure 9-4B.

9.4 Discussion

FNC is highly dynamic, even in the absence of external input [117]. Unlike conventional static FNC, which is obtained from the correlation within an entire time series, dFNC refers to the connectivity between any pair of brain regions within sub-portions of time series [117]. It is likely that any cognitive deficits and clinical symptoms associated with a particular brain disorder depend not only on the strength of the connectivity between any pair of specific brain regions but also on dynamic fluctuations of connectivity among those regions. Therefore, in this study, we hypothesized that studying VSN dFNC can elucidate the mechanism of deficits in visual regions in schizophrenia.

We collected rs-fMRI scans with closed eyes in schizophrenia and healthy individuals, along with clinical and visual learning performance data assessed by CMINDS to investigate visual network functional connectivity differences between these two groups.
This is, to the best of our knowledge, the first study that explored the dynamic functional network connectivity within the VSN, including middle temporal, middle occipital, calcarine, inferior occipital, lingual, fusiform gyri, and cuneus, and its association with visual learning in SZ subjects. We used a sliding window to calculate VSN dFNC over time and a k-mean clustering method to partition VSN dFNC into five connectivity patterns with unique fluctuations (states). Next, we compared the OCR for each state between SZ and HC.

By comparing FNC in different states, we found that the FNC between the middle temporal gyrus and other regions was highly negative in state 1 and state 2. However, the connectivity between this area and other regions was more positive in state 4. In addition, the connectivity among inferior occipital and middle occipital gyri was highly positive in states 1, 2, 4, and 5 with the exception of state 3. Also, middle occipital and fusiform gyrus connectivity with the rest of VSN was strongly negative in states 3 and 5, respectively. All of these observations suggest that the VSN functional connectivity is highly dynamic, even in the absence of external stimuli. This dynamic connectivity of VSN has not been explored in prior studies.

We also compared the VSN connectivity between HC subjects and SZ subjects in each state. Four states out of five states showed similar differences between SZ and HC subjects. In all states, the connectivity of cuneus in HCs was significantly higher than that in SZ subjects. This may indicate a central role of the cuneus in differentiating SZ subjects from healthy individuals. It is worth noting that the cuneus, which is involved in basic visual processing, has been shown to differentiate schizophrenia from healthy subjects quite consistently. Consistent with our results, several studies reported a lower cuneus
functional connectivity and altered structural properties in SZ compared to HC [243]–[245].

The correlation between the middle temporal gyrus and other regions was significantly higher in HC in all states except state 3. However, within-middle temporal gyrus correlation was significantly higher in HC subjects in all states. The middle temporal gyrus is involved in various cognitive processes, including semantic memory, visual perception, and sensory integration. A study in macaques suggested the role of the middle temporal gyrus in visual connection associated with object vision. The same study showed that ablation of this area causes a learning deficit in visual object discrimination and recognition [246]. In another study, higher activation of the middle temporal gyrus was linked to perceiving facial attractiveness and expression [247]. Several studies have reported functional and structural differences in the middle temporal gyrus between SZ and HC [248]–[252]. Middle temporal gyrus gray matter volume may be smaller in SZ subjects with first-episode schizophrenia [248] relative to controls. Another study reported smaller middle temporal gyrus gray matter in SZ subjects and their unaffected sibling compared to healthy subjects [250]. Our findings of lower connectivity within-middle temporal gyrus regions and between middle temporal gyrus and other regions of VSN in schizophrenia highlighted the importance of the role of the middle temporal gyrus in differentiating schizophrenia subjects from healthy individuals. While the cuneus shows a consistent pattern in all states, the middle temporal gyrus contributed differently across different states. This highlights another benefit of analyzing the VSN functional network connectivity at a shorter timescale [253].
Another area showing significantly lower functional connectivity in SZ is the calcarine sulcus area. This difference was more marked in states 1 and 4. Therefore, the calcarine sulcus may play a notable role in the pathophysiology of schizophrenia, as it displayed a lower gray matter volume of calcarine gyrus in SZ subjects [243]. In addition, we found significantly lower functional connectivity of the inferior occipital gyrus and fusiform gyri in SZ subjects. Less fusiform gray matter volume and thickness have been reported in schizophrenia (Lee et al., 2002; Onitsuka et al., 2003; Van Erp et al., 2018). Also, previous literature has highlighted the role of the inferior occipital gyrus and fusiform gyrus in face processing and facial recognition [257]–[259]. Neuroimaging studies show more activation of the fusiform gyrus in viewing face stimuli compared to nonface stimuli such as objects [260]. Previous studies reported abnormal face detection in schizophrenia [261]. Our result can potentially highlight the role of these regions in explaining some underlying mechanisms of face processing deficits of schizophrenia and suggest further study of the role of the inferior occipital gyrus and fusiform gyrus in the face-processing deficit of schizophrenia subjects. Besides, we observed a significant difference between HC and SZ in the shorter timescale connectivity of inferior occipital, middle occipital, and fusiform gyri. It is possible that additional study of the connectivity of these regions will reveal further underlying mechanisms of visual processing deficits in schizophrenia [257], [258].

By comparing the OCR of SZ vs. HC in each state, we found SZ subjects spent more time in state 1, showing highly negative connectivity of the middle temporal gyrus with other regions of VSN. The role of dFNC temporal pattern has been reported in several disorders, including schizophrenia, major depressive disorder, and Alzheimer’s disease [15], [19], [27], [119]. Our finding is consistent with previous results indicating broadly and
significantly suppressed whole-brain connectivity dynamic in SZ subjects and longer periods of weak connectivity among SZ subjects relative to controls [15], [19]. A previous whole-brain dynamic connectivity analysis showed that schizophrenia subjects spend less time in a highly-connected state [19]. Another study from our group showed an abnormal pattern in the dFNC of the default mode network (DMN) by comparing state-based connectivity strength, dwell time and between-state transition number of HC and SZ subjects [21]. This study identified an SZ-associated pattern in the temporal dynamics of DMN in SZ subjects by showing that they spend more time in a state with sparsely connected nodes. Also, this study demonstrated a state-specific spatial disruption within DMN by showing that the central hubs of the posterior cingulate cortex and anterior medial prefrontal cortex are significantly impaired in SZ subjects.

Finally, using the Pearson correlation between OCR and visual learning memory score in SZ subjects, we found a significant positive correlation with the OCR of state 4. Thus, SZ subjects with higher visual learning memory scores spent more time in this state. In addition, state 4 showed positive connectivity within the middle temporal gyrus and between the middle temporal gyrus with other regions, including inferior occipital, middle occipital, and fusiform gyri. This provides further evidence that the middle temporal gyrus has a significant role in dissociating SZ and HC and may also play a role in schizophrenia-associated visual learning abnormalities.

9.4.1 Limitations and future work

We have some limitations in this study as a previous study showed that some other factors, including dietary intake and nutritional status, drug usage, lifestyle, and even habitat, might
have an effect on cognition regardless of illness pathophysiology. For example, a recent study showed that the psychopathology on a variety of measures was reduced in those schizophrenia subjects who took vitamins C, and E with n-3 PUFAs EPA and DHA for four months [262]. Another study showed that schizophrenia subjects living in urban habitats showed more cognitive dysfunction than those who were not living in an urban environment [262]. However, these factors might have an effect on healthy subjects’ cognitive function as well [263]. To make sure all subjects are on the same level of intelligence, we exclude those subjects with IQs less than 75. We also believe that our large sample size (N>300) can reduce the confounds in our study.

In addition, we could not quantify whether the participant closed their eyes or stayed awake during the entire time of scanning, and it is based on the self-response of the subject after scanning. As a previous study showed, these might confound the results [264]. However, we assume that our dynamic functional connectivity approach would be able to separate those states in which the eyes are open, or the subject are not awake from other states. That potentially proved another benefit for dynamic functional connectivity analysis, which can separate undesired states (i.e., open eyes or sleep) from the desired ones (i.e., closed eyes and awake).

Although previous studies showed that the visual sensory network has a significant contribution to visual learning [265], other studies provide information about the contribution of other brain regions, such as the hippocampus, as a part of the cognitive control network, in visual learning. In addition, a previous study showed a dysconnectivity between VSN and other brain networks in schizophrenia subjects [19], [235]. Therefore, a prospective study on the effect of this dysconnectivity between brain networks on visual
learning might provide new information about visual learning impairment in schizophrenia subjects.

In addition, VSN might contribute to other cognitive tasks, including visual working memory [266], speed of processing [267], and visual attention [267]. As a previous study showed, these cognitive tasks might be impaired in schizophrenia subjects [236]. Therefore, future studies are needed to explore the link between VSN dysconnectivity and the aforementioned cognitive tasks.

9.4.2 Conclusion

Previous studies have shown visual processing deficits in schizophrenia. Here, we investigated the dynamic functional connectivity in the resting brain’s visual sensory network. By analyzing the visual network dFNCs and clustering them into five reoccurring states, we found, as expected, that this network is highly dynamic. By comparing the VSN between HC subjects and SZ subjects in each state, we found that SZ subjects show reduced connectivity in these regions in many states. In particular, we observed that the middle temporal gyrus (in four states), cuneus (in five states), calcarine gyrus, and fusiform gyrus display decreased connection in SZ. These findings suggest a substantial dynamic disruption within the visual system in the shorter timescale in SZ subjects. In addition, we found that in transient shorter timescale estimates, SZ individuals spend more time in a state with negative functional connectivity between the middle temporal gyrus and other areas. We also found that staying in a state with higher functional connectivity within and between the middle temporal gyrus and other areas positively correlated with visual learning scores in SZ subjects.
CHAPTER 10. ABERRANT DYNAMIC FUNCTIONAL CONNECTIVITY OF DEFAULT MODE NETWORK PREDICTS SYMPTOM SEVERITY IN MAJOR DEPRESSIVE DISORDER

10.1 Introduction

Major depressive disorder (MDD) is a serious mood disorder characterized by feelings of sadness, anger, loss, diminished interests, and social withdrawal [23], [24]. MDD affects more than 16 million, or 6.7 percent, adults in the United States and 350 million, or 4.4 percent, adults worldwide each year [25]. Despite significant progress in treating MDD, 20% to 30% of patients are treatment-resistant [26]. To improve treatments, we need a better understanding of the underlying mechanisms of MDD. In recent decades, functional network connectivity (FNC) studies based on resting-state functional magnetic resonance imaging (rs-fMRI) [268] have been used to reveal new information about the neurophysiological substrates of MDD by identifying abnormal communication within and between functional brain regions and networks [88], [269]–[274].

The default mode network (DMN) - which includes the anterior cingulate cortex (ACC), posterior cingulate cortex (PCC), precuneus (PCu), medial prefrontal cortex (mPFC), and lateral and inferior parietal cortex, has been of particular interest due to its highest engagement during the task-free resting state and its possible role in revealing information about the intrinsic brain [275]. Early investigations highlighted the role of DMN in spontaneous and task-unrelated thought during rest in the healthy subjects [276], and later studies showed its impairment in the self-referential process of MDD patients [277]. Also, it is shown to have a putative role in the generation of negative rumination and depressive
symptoms in MDD patients [278], [279]. [278] found an increasing level of activity in DMN than the task-positive network in MDD patients and its association with higher levels of depressive rumination and lower levels of reflective rumination. Another study predicted the suicidal behavior of depressed patients based on abnormal DMN connectivity [280]. All of these studies proved an essential role of DMN connectivity in MDD and highlighted its role in the pathology of depression.

Studies of the functional connectivity within DMN subnodes have reported inconsistent results regarding the activity of this network in MDD. While multiple studies reported increased DMN connectivity in MDD [278], [279], [281]–[284], there have also been other studies showing decreased functional connectivity of DMN subnodes in MDD patients [141], [285]. By assuming that FNC is static over time, the majority of these studies ignore the time-varying behavior of DMN FNC in MDD. Although FC is highly dynamic even in the absence of external inputs, DMN dFNC of MDD has not yet been comprehensively explored. dFNC has been studied in other disease groups [4], [117], [208], [216], [225].

To date, only three studies have assessed dFNC in MDD [27]–[29], [286]. Only one of those investigated the alteration of functional connectivity in DMN (between PCC and mPFC) by looking at the standard deviation of dynamic functional connectivity, though this was within a relatively small dataset [29]. Since it is well known that the brain is highly dynamic, we hypothesized that a focus on the dynamics among subnodes of the DMN network would reveal new information which cannot be found through whole-brain connectivity [287]. In addition, to avoid the strong assumptions of a seed-based approach to extracting the brain network components (regions), we used a semi-blind adaptive framework called NeuroMark [98]. NeuroMark provides a fully automated independent
component analysis (ICA) framework using spatially constrained ICA to estimate features adaptable to each individual and comparable across subjects by taking advantage of the replicated brain network templates extracted from two N~900 normative resting fMRI data sets. We obtained seven data-driven DMN subnodes, including ACC (two nodes), PCC (two nodes), and PCu (three nodes), based on the NeuroMark template and showed an abnormal temporal pattern and a link between this abnormal connectivity pattern and symptom severity in MDD patients.

In this chapter, we leveraged the sliding window approach followed by k-means clustering to identify a set of connectivity states to investigate dFNC within the DMN in MDD [117]. To further investigate and model the temporal changes in dFNC, we estimated a hidden Markov model (HMM) transition probabilities between dFNC states. Next, via statistical analysis of the estimated HMM features, we explored a link between symptom severity and abnormal DMN dFNC in MDD. Also, we leveraged a machine learning approach to investigate the DMN subnodes connectivity differences between MDD and healthy control (HC) subjects in each estimated state.

10.2 Materials and methods

In this study, ethical approval was granted by the relevant ethics committees, and informed consent was obtained from each subject prior to scanning according to each site's institutional review board.

10.2.1 Participants

Data were collected from 539 Chinese Han participants (262 MDD patients and 277 HCs) that were recruited from 4 hospitals in China, including the West China Hospital of Sichuan (Site 1), the Henan Mental Hospital of Xinxiang (Site 2), the First Affiliated Hospital of
Zhejiang (Site 3), and the Anding Hospital of Beijing (Site 4). The structured clinical interview for diagnostic and statistical manual of mental disorders (SCID-P) confirmed MDD diagnosis, and SCID/NP confirmed the absence of a psychiatric diagnosis for HC. In addition, HCs with any psychiatric disorder history in their first-degree relatives were excluded. The 17-item Hamilton depressive rating scale (HDRS) assessed the current symptom severity of MDD subjects [288].

10.2.2 Imaging protocol

In site 1, a 3T Philips scanner (Achieva, Netherlands) with an 8-channel phased-array head coil was used for collecting the functional images. Repetition time (TR)/echo time (TE) = 2,000/30 ms, field of view (FOV) = 240 × 240 mm (64 × 64 matrix), flip angle (FA) = 90°; 38 sequential ascending axial slices of 4 mm thickness and 0 mm gap were the parameters used in this process. For site 2, a total of 240 volumes of echo-planar images were collected using a 3T Siemens scanner (Verio, Germany) with a 12-channel phased-array head coil and TR/TE = 2,000/30 ms, FOV = 220 × 220 mm (64 × 64 matrix), FA = 90°; 33 sequential ascending axial slices of 4 mm thickness and 0.6 mm slice gap. Site 3 used a 3T Siemens scanner (Prisma, Germany) with a 12-channel phased-array head coil for acquiring the fMRI data. The scanning parameters were TR/TE = 2,000/30 ms, FOV = 220 × 220 mm (64 × 64 matrix), FA = 90°, 38 sequential ascending axial slices of 4 mm thickness and 0 mm slice gap. For site 4, a 3T Siemens scanner (Prisma, Germany) with a 32-channel phased-array head coil was used. In this site, the scanning parameters were TR/TE= 2000/30ms, FOV=220 × 220 mm (64 × 64 matrix), FA=90°, 38 sequential ascending axial slices of 4 mm thickness and 0.7 mm gap. During scanning, foam padding and earplugs
were used to minimize head movement and scanner noise, and subjects were instructed to keep their eyes closed and stay awake during the resting-state scan.

10.2.3 Data processing

The preprocessing and extracting the independent components from DMN are described in Chapter 8.

10.2.4 Dynamic functional network connectivity (dFNC)

For each subject, $i = 1 \ldots N$, dFNC of the seven subnodes of DMN was estimated via a sliding window approach. A tapered window, which was obtained by convolving a rectangle (window size = 20 TRs = 40 s) with a Gaussian ($\sigma = 3$), was used to localize the dataset at each time point. The correlation matrix was estimated using the windowed data to measure the dFNC between seven subnodes in DMN. Twenty-one connectivity features were estimated out of seven subnodes in DMN. We concatenated dFNC estimates of each window for each subject to form a $(C \times C \times T)$ array (where $C=7$ denotes the number of DMN subnodes, and $T=205$ denotes the number of windows), which represented the changes in brain connectivity between DMN subnodes as a function of time [4], [117], [216].

10.2.5 Clustering and latent transition probability feature estimation

The method is the same as the method described in Chapter 8.

10.2.6 Quantifying group difference using the feature selection method

To quantify the DMN FC difference between MDD and HC in each state, a logistic regression (LR) was used to perform a classification between those two groups based on their connectivity features, which are twenty-one features (i.e., $C_1$, $C_2$, $\ldots$, $C_{21}$) in total as shown in Figure 8-2. It worth mentioning that with seven subnodes in DMN, we estimated
twenty-one connectivity features in which each feature represents the connectivity strength between any pair of DMN subnodes. In opposition to statistical learning, which looks at each feature at a time and does not consider the interaction between input features, the machine learning-based feature selection approach would give a generalized model of the difference between HC and MDD features [103]. We used leave-one-site nested cross-validation (CV) in which all subjects from one site were used for the test and the other three sites for training [218]. We used this approach for training and testing to evaluate the effect scanning site, and with four sites, we did this process four times. In the nested CV, the data were divided into training and test sets in any outer fold. Then, the training data was divided into another training and validation data in an inner fold. The optimized parameters have been identified by training different models using inner-loop training data and validated using the validation data set. In this process, the hyperparameters of each model are tuned to minimize the inner-fold CV error of the generalization performance. The details of the method are provided in Chapter 8.

10.2.7 Statistical analysis

To find a link between HMM features and HDRS of the MDD group, we used a partial correlation with the Pearson method and by accounting for the age, gender, and scanning sites. We performed all statistical analyses on all twenty-five HMM features. For the false discovery rate (FDR) correction, all p values have been adjusted by the Benjamini-Hochberg correction method. Also, to find the most important feature in ENR, we used multiple comparisons on a one-way ANOVA test [289].
### Table 10-1 Demographic and clinical information of the participants in each site

<table>
<thead>
<tr>
<th>Site</th>
<th>Number</th>
<th>MDD</th>
<th>HC</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>70</td>
<td>70</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Age (year: Mean±SD)</td>
<td>33.29±10.63</td>
<td>33.24±10.48</td>
<td>0.95</td>
</tr>
<tr>
<td></td>
<td>Gender(M/F)</td>
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<td>25/45</td>
<td>0.99</td>
</tr>
<tr>
<td></td>
<td>HDRS (Mean±SD)</td>
<td>23.35±7.17</td>
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<td>NA</td>
</tr>
<tr>
<td></td>
<td>duration of illness (month: Mean±SD)</td>
<td>38.13±50.63</td>
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<td>NA</td>
</tr>
<tr>
<td>2</td>
<td>85</td>
<td>68</td>
<td>NA</td>
<td></td>
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<tr>
<td></td>
<td>Age (year: Mean±SD)</td>
<td>35.43±12.87</td>
<td>35.92±12.73</td>
<td>0.73</td>
</tr>
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<td></td>
<td>Gender(M/F)</td>
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<td>30/38</td>
<td>0.99</td>
</tr>
<tr>
<td></td>
<td>HDRS (Mean±SD)</td>
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<td>NA</td>
</tr>
<tr>
<td></td>
<td>duration of illness (month: Mean±SD)</td>
<td>84.63±95.19</td>
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<td>NA</td>
</tr>
<tr>
<td>3</td>
<td>29</td>
<td>29</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Age (year: Mean±SD)</td>
<td>35.13±9.02</td>
<td>29.89±7.34</td>
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</tr>
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<td></td>
<td>Gender(M/F)</td>
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<td>12/17</td>
<td>0.99</td>
</tr>
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<td>HDRS (Mean±SD)</td>
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<td>NA</td>
</tr>
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<td>duration of illness (month: Mean±SD)</td>
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<td>NA</td>
</tr>
<tr>
<td>4</td>
<td>29</td>
<td>29</td>
<td>NA</td>
<td></td>
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<tr>
<td></td>
<td>Age (year: Mean±SD)</td>
<td>32.91±11.07</td>
<td>31.05±10.66</td>
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</tr>
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<td></td>
<td>Gender(M/F)</td>
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<td>101/176</td>
<td>0.68</td>
</tr>
<tr>
<td></td>
<td>HDRS (Mean±SD)</td>
<td>19.32±7.35</td>
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<td>NA</td>
</tr>
<tr>
<td></td>
<td>duration of illness* (month: Mean±SD)</td>
<td>51.81±69.15</td>
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<td>NA</td>
</tr>
<tr>
<td>Total</td>
<td>262</td>
<td>277</td>
<td>NA</td>
<td></td>
</tr>
</tbody>
</table>

**Note:** MDD: Major depressive disorder; HC: healthy control; HDRS: Hamilton depression rating scale; SD: standard deviation; NA: not applicable; all p values have been calculated using two-sample Kolmogorov-Smirnov test.

#### 10.3 Results

##### 10.3.1 Demographic and clinical characteristics

The site-based demographic and the clinical characteristics of the participants are shown in Table 10-1. Using a Kolmogorov–Smirnov two-sample test on all subjects combining all sites, we did not find a significant group difference between HC and MDD in age and gender. However, the age difference between MDD and HC subjects was significant within
Across all MDD patients, the mean and the standard deviation of HDRS were 19.32 and 7.35, respectively.

10.3.2 The dynamic connectivity states

Five different DMN dFNC states identified by the k-means clustering are shown in Figure 10-1. We observed different connectivity patterns in these five states. State 2, 4, 5 showed more positive connectivity in PCu regions. However, we observed less connectivity in three regions of PCu in state 1, and 3. State 4 was the only one that showed negative connectivity in ACCs. Also, states 2, 3, and 4 showed positive connectivity at PCCs, and other states showed negative connectivity in these subnodes of DMN. State 2 was the only one that showed less connectivity between ACC and other regions in DMN. In addition, states 2 and 4 showed more positive connectivity between PCu and PCC. However, other states showed both positive and negative connectivity between PCu and PCC.

10.3.3 Difference between MDD and HC connectivity in each state

We used the connectivity features, in total, twenty-one features obtained from seven subnodes of DMN to compare HC and MDD in each state. Each feature showed the strength of the connectivity between any pair of DMN subnodes. We used a 4-fold (leave-one-site-out) CV LR classifier with the ENR feature learning method to model the difference between HC and MDD groups in each state. Figure 10-2A shows the feature
The normalized occurrences of connectivity feature in this classification are shown in this figure. Features retained by the ENR that were significantly more frequently than the average inclusion rate are shown in purple. As this figure shows, $C_{11}$, $C_{15}$, $C_{16}$, and $C_{18}$ were equally the most important features in the classification between HC and MDD in this state. In addition, Figure 10-2A (right panel) shows the group differences between MDD patients and HCs in the strength of those connectivity features selected by ENR. In this figure, the red lines represent increased connectivity, and the blue lines represent decreased connectivity in MDD patients compared to HCs. In addition, the
wider line means larger group differences. As this figure shows, HCs had stronger connectivity between PCu (including PCu2 and PCu3) and PCC. Also, the connectivity between ACC1 and ACC2 was less in HCs than that in MDD subjects in this state. In addition, the connectivity between ACC and PCC was higher in HCs than that in MDDs. Based on the feature selection results, we did not observe any significant difference between MDD and HC in the connectivity within PCus and within PCCs.

The normalized occurrences of connectivity features in the classification between HC and MDD of state 2 are shown in Figure 10-2B. In this classification, using a 4-fold CV, the mean value of the classification AUC was 0.64. As this figure shows, the contribution of $C_{10}$, $C_{11}$, and $C_{14}$ was significantly and equally higher than other features. Also, we found that the connectivity between PCu2 and PCC2, and the connectivity between PCu3 and PCC1 were higher in HC subjects. While the connectivity between PCC1 and PCu2 was lower in HC subjects. Therefore, overall, we observed more connectivity between PCu and PCC in HC than MDD subjects in this state. No significant difference between MDD and HC was observed in within-region connectivity in this state. Interestingly, compared with other states, no significant difference between ACC nodes was observed in this state.

The feature selection result in the classification between HC and MDD of state 3 is shown in Figure 10-2C, where the AUC of classification was 0.58 in a 4-fold CV. In this classification, the $C_1$, $C_2$, $C_{14}$, $C_{16}$, and $C_{18}$ were equally the most important features, differentiated between HC and MDD. In this state, we observed weaker connectivity within PCu (i.e., PCu1, PCu2, and PCu3) regions and within ACC (i.e., ACC1 and ACC2) regions in HC subjects. Also, we found stronger connectivity between the connectivity between PCu3 and PCC1 and between ACC1 and PCC2 of HC subjects in this state. This was the
only state that showed a difference between MDD and HC subjects in the connectivity among PCu nodes.

**Figure 10-2D** shows those features that were retained by the ENR equally and significantly more frequently than other features in the classification between HC and MDD in state 4. In this classification, the mean AUC of 4-fold CV was 0.57, and \( C_{11}, C_{16}, \) and \( C_{17} \) had the strongest contribution compared to other features. In this state, the connectivity within ACC regions and between PCu2 and PCC2 was higher in HC subjects. Also, the connectivity between ACC1 and PCC1 was less in this group. This state is the only state that showed higher connectivity within ACC nodes in HC subjects.

Finally, the result of feature learning in the classification between HC and MDD of state 5, in which the mean value of 4-fold CVAUC was 0.59, is shown in **Figure 10-2E**. Among
all connectivity features, only $C_5$, $C_{11}$, and $C_{16}$ showed significant and equal contributions in this classification. In state 5, the PCu and PCC connectivity was stronger in HCs, and the connectivity of ACC was weaker in these subjects.

10.3.4 Behavioral correlation with HMM features

The next important question was how DMN between-state transition features (or HMM) are correlated with symptom severity. To answer this question, we calculated the Pearson's linear correlation between transition probability, i.e., $a_{ij}$, and HDRS while controlling for age, gender, and scanning site. The correlation between HDRS and state transition probability and their associated FDR corrected $p$ values are shown in Figure 10-3A. With twenty-five HMM features, we had twenty-five correlation and their associated $p$ values, as shown in this figure. Based on this analysis, only one correlation between HDRS and the state transition feature was significant after correcting for multiple comparisons (FDR corrected $p < 0.05$). We found that symptom severity showed a positive correlation with state transition from state 4 to state 3 ($r=0.19$, FDR corrected $p=0.04$, $N=234$), as shown in Figure 10-3B. In other words, the transition from state 4, which showed higher connectivity between PCu and PCC and lower ACC connectivity, to state 3, with lower connectivity between PCu and PCC and higher ACC connectivity, increased by symptom severity. It is worth mentioning that there are subjects that do not have any transition from state 4 to state 3, as shown in Figure 10-3B. To explore whether these subjects would drive the result or not, we removed these subjects and repeated the correlation analysis. As expected from the graph, after removing those subjects, the correlation values increased and became more significant. The corrected $p$ value changed from ($r=0.19$) 0.04 to ($r=0.33$)
Overall, removing those subjects with zero transition probability increases the correlation between the transition from state 4 to state 3 with symptom severity.

10.4 Discussion

In this study, we investigated the differences in functional connectivity dynamics during rs-fMRI between HC individuals and individuals with MDD using a relatively large sample size. To this end, we framed our approach around three main questions; 1- what the dFNC reoccurring patterns are across time and across subjects? 2- What is the difference between dFNC of MDD and HC subjects in each of these patterns? 3- How do the temporal properties of these patterns correlate with symptom severity?

For the first question, we found a disrupted pattern within and between the connectivity of PCu, ACC, and PCC subnodes. We observed both positive and negative connectivity in PCC and ACC. Also, within PCu, connectivity was stronger in states 2, 4, and 5 than that in states 1 and 3. Our work goes beyond previous research by incorporating the dynamics of DMN FC using data-driven subnodes and shows that functional connectivity within DMN subnodes is indeed highly dynamic, representing a higher activity and flexibility in functional coordination in this mode. While at least one previous study has evaluated DMN dynamics using pre-defined regions of interest [29], there has not yet been an approach that utilized data-driven DMN subnodes and compared the state connectivity difference between patients and controls and linked the between-state transition probability with symptom severity. As recent work has highlighted, it is important to ensure the data within the node is consistent, or results can be degraded or misleading [220] or inconsistent [141]. This is especially true when studying dynamics [127].
Based on static functional connectivity analysis in DMN, previous literature has reported inconsistent results within DMN subnodes connectivity by showing both increases [278], [279], [281]–[284] and decreases [141], [285], and even no significant difference between MDDs and HCs [290] in the connectivity of this network. Although this inconsistency could in part be due to differences in disease subtypes or symptoms and even small sample size as [141] claimed, we hypothesize that the heterogeneity is partially driven by the focus on static functional connectivity, which is obtained from the correlation within an entire time series. The current study, which showed a disrupted (both increase and decrease) pattern within DMN connectivity, provides a more natural analytic approach, enabling us to focus on the dynamics of the network over a shorter time span. In addition, as [141] claimed, another reason for having inconsistent results in comparing the DMN connectivity between MDD and HC in the previous literature is using different preprocessing parameters. Our usage of Neuromark, a replicable platform for extracting the subnodes within DMN, was developed to address this issue [98].

We cast the second question into a classification problem to differentiate between HC and MDD subjects in each state. We trained an LR with ENR as an embedded feature learning to find the most important connectivity features in the classification between HC and MDD subjects. Using the feature learning method, we found that the connectivity between PCu and PCC is one of the most important features that differentiate between HC and MDD in all states. Also, we found that the connectivity between PCC and PCu is relatively lower in MDD patients than that in HC subjects. Previous studies showed more activation in PCu, and PCC plays an important role in self-reflective thinking, which is the main feature of depression [291], [292]. In another study, the activity in PCu and PCC was decreased
by disrupting normal neural circuitry in the medial parietal region using transcranial magnetic stimulation, and this caused a decrease in self-references [293]. In addition, more activation in PCu/PCC has been reported during the evaluation of self-referential pleasantness [294]. Also, a previous study reported lower functional connectivity between PCC and PCu in the first episode of treatment-naïve patients [274]. By analyzing the dFNC of DMN, the current study offers new evidence on the connectivity between PCu and PCC in MDD subjects and further supports the role of the connectivity between PCC and PCu in the pathogenesis in MDD.

Also, results showed that ACC connectivity (i.e., the connectivity between ACC1 and ACC2) contributes to the classification between MDD and HC in all states except state 2. Based on the anatomical template we used [295], we found that ACC1 is posterior ACC or pACC, and ACC2 is subgenual ACC or sgACC. The ACC, which is connected with both the emotional limbic and the cognitive prefrontal cortex, is involved in the integration of neuronal circuitry for the management of uncomfortable emotions [189], [296]. Several years of studies proved a substantial role of ACC subregion, in particular sgACC, in the pathology of MDD. For many years sgACC has been the main deep brain stimulation target for producing prolonged remission from depression [297].

A previous study found ACC hyperactivity during sadness in healthy subjects [298]. Also, [279] reported a higher sgACC connectivity in MDD patients compared with HC subjects. A recent study reported a positive link between the connectivity of the sgACC and dorsal ACC (dACC) and the persistence of sadness and inflexibility of daily emotions in both HC and MDD subjects [299]. However, the same study also showed a reduced ACC connectivity in MDD vs. HC. In addition, [285] showed a decrease in sgACC connectivity
in MDD patients. These contradicting results are possibly due to focusing on static functional connectivity and ignoring the highly dynamic nature of these subnodes. In our current study, in three states out of five, we observed increased functional connectivity within ACC (i.e., between sgACC and pACC) in MDD subjects. In addition, we found that ACC connectivity is more in HC subjects in state 4, and this connectivity does not show a significant difference between MDD and HC in state 2. An aberrant spatiotemporal pattern in the connectivity between two ACC subregions potentially stressed the importance of studying dynamic functional connectivity and evaluating the connectivity in a shorter timescale. This abnormal pattern possibly can explain why previous studies based on static functional connectivity reported inconsistent (both increase and decrease) results within ACC connectivity in MDD vs. HC. Also, we found a disrupted spatiotemporal pattern in the connectivity between ACC1 (or pACC) and PCC by showing a lower MDD connectivity in state 1 and state 3 and higher MDD connectivity in state 4. In addition, no significant difference between MDD and HC is observed in state 2 and state 5. This disrupted pattern of pACC and PCC connectivity in the gap between MDD and HC potentially marks the importance of analyzing functional connectivity in a shorter period and suggests further prospective investigation in the connectivity between ACC and PCC in MDD.

For the last question, first, we estimated the HMM transition probability to model the temporal changes of dFNC. Then, using the Pearson correlation between HMM transition probability and HDRS, we found a significant positive correlation between the transition from a state with high PCu/PCC (the connectivity between PCu and PCC) and low ACC connectivity to a state with lower PCu/PCC connectivity and higher ACC and symptom
severity. These results provide more evidence about the role of connectivity between PCu and PCC and the connectivity in ACC as a biomarker of MDD, and this role is higher in severe MDD. Recent studies on Alzheimer’s Disease found an association between the number of transitions among states and symptom severity [119]. In the current study, we used between-state transition probability from HMM, which mathematically is different from the study mentioned above, and for the first time, we found a correlation between symptom severity of MDD and HMM features. In the current study, we introduced HMM features as a potential biomarker that possibly can elucidate some underlying mechanism in patient symptom variation and its association with the temporal pattern of DMN connectivity. Besides, quantification of the link between symptom severity and HMM features (an in the general dynamic pattern) in MDD patients potentially leads to an optimized treatment and also prognostic of MDD, which needs future investigation.

The default mode network (DMN) has been one of the main targets for deep brain stimulation (DBS) and repetitive transcranial magnetic stimulation (rTMS) for many years. For example, a recent study showed that rTMS reduces the functional connectivity within DMN [300]. On the other hand, the state-dependent stimulation showed more efficiency...
than the blind stimulation, in which we do not account for the state of the brain at the time of stimulation[301]–[303]. However, it remains unclear which biological properties should be used as the most appropriate control signal and what is the target brain state for stimulation [304]. In the current study, we introduce ACC connectivity and the connectivity between PCu and PCC as a potential marker to control and optimize the stimulation parameters. Our results suggest a possible benefit of administering the stimulation during the state with higher ACC connectivity and lower PCu/PCC connectivity and changing that state to a state with lower ACC and higher PCu/PCC connectivity (Figure 10-4). Also, the between-state transition probability is another marker that can be used as a control signal in the closed-loop stimulation. In a closed-loop therapy, we should reduce the transition probability from a state with lower ACC and higher PCu/PCC connectivity to another one with higher ACC and lower PCu/PCC connectivity. There are, of course, many technical limitations to implementing a real-time system that can calculate and find brain functional connectivity states and administering TMS while we collect fMRI data[155]. However, the results suggest a possible benefit of moving in this direction.

10.4.1 Limitations and future work

Although HDRS is widely used in scaling the symptom severity of depression, this score is highly dependent on the skill and knowledge of the interviewer [305]. Since the data in this study are coming from four different sites with different raters, this might add a variation and error for HDRS values across sites. In addition, this score is heavily focused on somatic symptoms, and previous studies questioned its reproducibility across studies [306]. The choice of window size is an implicit assumption about the dynamic behavior in
that a short window captures more rapid fluctuations, whereas a longer window does more smoothing than a shorter one. Future work can be accomplished to evaluate the range of dynamics more comprehensively [161]. In addition, prospective studies need to be done in different feature selection methods to validate the reproducibility of the feature learning results [307].

10.4.2 Conclusion

Previous studies of static FNC have shown that DMN plays an essential role in MDD. In the work reported here, we extend this existing body of knowledge into the dynamic realm, investigating how time-varying properties of DMN connectivity relate to MDD and its symptom severity. We found that in shorter timescale estimates, MDD patients exhibit lower connectivity between PCu and PCC and have long been associated with reflective thinking. Similarly, consistent with earlier static FC studies identifying a relationship between ACC connectivity and persistent sadness, in our time-resolved connectivity estimates, MDD patients exhibited elevated ACC connectivity. Furthermore, we found that patients with higher symptom severity are more likely to transition from a state with higher PCu/PCC (the connectivity between PCu and PCC) connectivity and lower ACC connectivity to a state with lower connectivity in PCu/PCC and higher connectivity in ACC. Ours is the first study DMN dFNC in a relatively large MDD sample that provides evidence of aberrant time-varying activity in DMN and demonstrates a link between this aberrant activity and the symptom severity in this disease group.
CHAPTER 11. DYNAMIC FUNCTIONAL CONNECTIVITY LINKS WITH TREATMENT RESPONSE OF ELECTROCONVULSIVE THERAPY IN MAJOR DEPRESSIVE DISORDERS

11.1 Introduction

Major depressive disorder (MDD) is a debilitating brain disorder [308], which is characterized by impaired cognitive functioning, somatic abnormalities such as inattention and inability to focus, as well as emotional troubles [309], [310]. There are effective treatments accessible such as psychotherapy and alleviating medications, but it has been declared that 30 percent of patients suffering from MDD do not respond to these treatments [311]. Therefore, there is an essential need for advanced therapies such as deep brain stimulation (DBS), transcranial magnetic stimulation (TMS), and electroconvulsive therapy (ECT), which are being broadly used as an alternative to alleviate MDD symptoms [312]–[314].

Among all mentioned therapies, ECT can be considered one of the most effective treatments for pharmacological resistant MDD [315] due to its faster action and higher remission rate than typical medicine-based treatments [316]. One hundred thousand annual ECT treatments in the U.S revealed that this treatment's success rate is around 75% [317]. It has been shown that most depressive episodes disappear after 3–4 weeks of ECT series [317]. Yet, underlying neural and cognitive mechanisms behind this improvement caused by ECT are still unclear. Moreover, whether we can predict the effectiveness of ECT before applying it remained open.
In recent years, functional network connectivity (FNC) data obtained from resting-state functional magnetic resonance imaging (rs-fMRI) time series has demonstrated highly informative about the underlying brain connectivity patterns in mental disorders such as MDD [53], [309], [318], [319]. Recently, studies have shown that ECT resets and stimulates the formation of the brain regions/networks connectivity [320]–[322]. An investigation of the whole-brain FNC of the patients with depression showed a reduction in the left dorsal lateral prefrontal cortex connectivity corresponding to ECT therapeutic course [323]. Several recent studies reported functional and structural connectivity changes that occurred in the amygdala and anterior cingulate cortex (ACC) after ECT [324]–[328]. Another study declared that the cognitive control network (CCN) and the default mode network (DMN) play a vital role as the most effective brain network in regulating brain connections after ECT [329]. Moreover, increased intra-network connectivity in CCN [320], [321], and decreased dorsolateral prefrontal cortex global functional connectivity (DLPFC) as a part of CCN [323], are reported to be occurred after applying ECT. Other rs-fMRI studies reported significant changes in functional connectivity of DMN by applying ECT [330]–[332].

In the studies mentioned above, FNC estimated at CCN and DMN is often assumed to be static over time. However, this assumption runs contrary to the dynamic nature of brain FNC, and dynamic FNC (dFNC) has been recently introduced to overcome this limitation [4], [333], [334]. In recent years, a few studies have looked into dFNC in MDD and discovered that MDD affects both the strength and the temporal properties of FNC. As a result, we hypothesized that looking at the effect of ECT on dFNC might reveal how and to what extent ECT affects DMN and CCN.
This chapter used rs-fMRI data from 119 patients with depression (DEP) who experienced a series of ECT and 61 healthy controls (HC) to find the neural mechanisms behind the improvement after ECT and predict the effectiveness of ECT before applying it. To this aim, we used group independent component analysis (ICA) and extracted independent components from DMN and CCN, and estimated dFNC in these two networks by applying a sliding window approach and clustered dFNC into a few brain states using k-means clustering. Finally, we compared the occupancy rate (OCR) estimated from the state vector, an output of k-means clustering, between HC and DEP in pre-and post-ECT. Moreover, by correlating OCR with clinical data, we could significantly predict the effectiveness of ECT by using just pre-ECT rs-fMRI data.

11.2 Materials and methods

11.2.1 Participants and clinical outcome

This study used neuroimaging, clinical, and demographic information of 119 patients (76 females) diagnosed with depression (called DEP hereafter) and 61 healthy (HC) participants (34 females) from either University of New Mexico (UNM) or the University of California Los Angeles (UCLA). Exclusion criteria were as follows: 1) Any neurodegenerative and neurological disorders such as Alzheimer's disease or psychiatric conditions such as schizophrenia.2) Having alcohol or drug addiction, pregnancy, and potential dangers under magnetic resonance imaging (MRI) such as using a pacemaker.

Hamilton Depression Rating Scale-17 items (HDRS) were used to assess the patient group's symptom severity before and after the ECT [335]. Initial and final assessments were given to participants before ECT started and within a week of completing the ECT series, respectively. Some participants from UNM were investigated to have psychotropic
medications initially, but further investigations on UNM and all UCLA participants who stopped revealed psychotropic medications before ECT outset. The demographical information and clinical measurements can be seen in Table 11-1. Finally, All the participants signed the consent form, and this study has been approved by the institutional review boards at UNM and UCLA.

11.2.2 ECT procedure

In the UNM site, Thymatron System IV (Somatics, Lake Bluff, IL, USA) was used, and the ECT procedure was initiated with a right unilateral d’Elia (ultra-brief pulse width of 0.3 ms, stimulus dosage at 6 × thresholds) placement of electrodes. If the participant did not respond to ECT, the treatment continued with bitemporal (brief pulse width of 1 ms, stimulus dosage at 2 × thresholds) electrode placement. In UCLA, a Mecta 5000Q (MECTA Corp., Tualatin, OR, USA), the exact electrode placement, and similar stimulus dosages are used in UNM. Treatments were applied three times a week until obtaining a stable clinical response or the psychiatrist decision to stop treatment in the context of nonresponse. The ECT implementation procedure followed the clinical standards announced by the APA ECT Task Force Report and was not manipulated for the goal of this study. Patients were oxygenated during the treatment process and received adequate induction (methohexital or etomidate) and relaxation (succinylcholine). Clinical measures such as blood pressure were monitored during the treatment.

11.2.3 fMRI data acquisition

At the UNM site, a 3-Tesla Siemens Trio scanner (Siemens Healthcare, Malvern, PA, USA) was used to collect MRI data. Parameters of the whole-brain gradient-echo echo-planar imaging sequence are as follows: echo time (TE) = 29 milliseconds (ms), repetition
time (TR) = 2 s (s), voxel size = 3.75 × 3.75 × 4.55 mm, flip angle (FA) 75°, and 154 volumes. At UCLA, a 3-Tesla MAGNETOM Allegra MRI scanner (Siemens, Erlangen, Germany) was used to collect MRI data. Parameters of functional images are as follows: TE = 30 ms, TR = 5 s, voxel size= 3.4 × 3.4 × 5 mm, FA = 70°, and 180 volumes. The duration of resting-state scans was a minimum of 5 minutes and 16 seconds, and participants were guided to passively keep their concentration to the fixation cross during the scan.

11.2.4 Data preprocessing

We applied the same preprocessing described in other chapters.

The Neuromark fully automated group ICA pipeline using GIFT (http://trendscenter.org/software/gift) is implemented to extract reliable CCN and DMN independent components (ICs) shown in Figure 2-2 and Table 2-1.

11.2.5 Functional network connectivity

A sliding window which is a convolution of a rectangle (window size = 20 TRs = 40 s) with a Gaussian (σ = 3s), is applied to the data to calculate DEP. This method was used to localize the dataset per time point, and the procedure can be seen in Fig1 Step1. Since the neuroimaging data have different temporal resolutions (TR = 2 s for UNM and 5 s for UCLA), the individual data with the low temporal resolution was interpolated based on the high temporal resolution data to construct new TCs. Such an interpolation strategy has been successfully introduced in previous dFNC studies, showing reliable performance for capturing FNC dynamics in datasets with different temporal resolutions [336], [337]. Next, we used the Pearson correlation method to calculate dFNC between 24 sub-nodes of DMN and CCN. Then, we obtained 276 connectivity features (Fig1 Step1). Calculated dFNC for
each window was concatenated for each individual as a form of the $C \times C \times T$ array (where $C$ is the number of ICs and equals 276, and $T$ represents total windows and equals 610). Finally, all arrays for all participants were concatenated to show brain connectivity changes between ICs as a function of time [4], [338], [339].

11.2.6 Clustering and dFNC latent features

We implemented a K-means clustering method on the previous step's output. First, we concatenated dFNCs of all participants, then we used a k-means clustering method to put them into a few clusters or states [4], [333]. We used the elbow criterion to calculate the optimum number of clusters (optimum k in the k-means method), a clustering analysis standard [333]. This method defines the optimization equation as the distance of within-cluster and between clusters as a ratio and tries to minimize this ratio. We found the optimal number of clusters is 3, searching from $k=2$ to 9. We used the correlation as our distance metric with 1000 iterations. This process yielded 3 distinct states for the group of participants and the state vector for each individual. State vector shows the state of the brain at any given time. Subsequently, based on the state vector, we calculated each subject's time interval (the number of time windows that each participant was in a specific state), and we call this feature the OCR of each state. Thus, considering three states, we have three OCRs for each individual. Finally, we calculated the traveled distance for each participant using Euclidean distance. To determine the distance traveled, we calculated the distance between each time window of dFNC matrix and then summed up all possible window pairs' distances. So, we have one traveled distance for each individual, and it is a state-independent metric.

11.2.7 Statistical analysis
The OCR feature and traveled distance between DEP and HC group are compared using two samples t-test. Moreover, to see whether OCR can predict HDRS scores, partial correlation accounting for age, gender, number of treatments, and scanning site is used. All p values were adjusted by the Benjamini-Hochberg method for false discovery rate or FDR.

11.3 Results

This section discusses the results obtained from dFNC analysis and compares DEP and HC groups. It consists of dFNC states resulting from clustering analysis, the correlation of OCR with HDRS scores, a comparison between DEP and HC in OCR both in pre-ECT and post-ECT, and the traveled distance between two groups.

11.3.1 Clinical results

The clinical and demographical information of participants is provided in Table 11-1 separately for DEP and HC groups. Concerning the HDRS scores, the DEP group has an average of 25.59 and a standard deviation of 6.14 in pre-ECT, an average of 11.48, and a standard deviation of 9.07 in post-ECT. Using a two-sample t-test, we found a significant difference (p<0.00) between HDRS values of pre- and post-ECT values. In other words, the clinical scores showed that after the implementation of ECT, the HDRS scores had significantly decreased.

11.3.2 Dynamic functional network connectivity states for pre-ECT and post-ECT

We found three separate clusters (states) applying the k-means clustering method on dFNC of all individuals (both DEP and HC group). It is worth mentioning that we applied the clustering method to pre-ECT and post-ECT dFNC data separately. Figure 11-1A and Figure 11-1B show these three distinct states for pre-ECT and post-ECT, respectively. We
found pre-ECT and post-ECT rs-fMRI generate similar brain states. To assess this similarity across corresponding states, we used Fisher correlation coefficients (state1: $R=98.47\%$, state2: $R=87.5\%$, state3: $R=97.67\%$). Additionally, we calculated the average dFNC values of CCN, DMN, and CCN/DMN (i.e., the connectivity between DMN and CCN), as shown in Table 11-2.

We found that both state 2 and state 3 have higher within-CCN connectivity than state 1 in pre-ECT (state 1-2: corrected $p=0.02$, and state 1-3: corrected $p=0.04$). While state 1 had more increased within-DMN connectivity than the other two states in both pre-ECT and post-ECT (in pre-ECT: corrected $p$ (state 1-2) < 0.0001, and corrected $p$ (state 1-3) < 0.0001. In post-ECT: corrected $p$ (state 1-2) < 0.0001, and corrected $p$ (state 1-3) < 0.0001). Only state 2 showed positive functional connectivity between DMN and CCN in both pre-ECT and post-ECT data.

| Table 11-1 Demographic and clinical details of participants for each site. |
|-----------------------------------|----------------|----------------|---|
|                                  | DEP            | HC             | P-value |
| TCLA | Number       | 45             | 33             | NA |
|      | Age          | 41.22±13.51    | 39.03±12.21    | 0.46 |
|      | Gender(M/F)  | 20/25          | 15/18          | 0.99 |
|      | Pre-ECT HDRS | 2517±6.15      | NA             | NA |
|      | Post-ECT HDRS| 16.22±9.33     | NA             | NA |
| UNM | Number       | 74             | 28             | NA |
|      | Age          | 64.99±9.09     | 60.22±8.02     | 0.02 |
|      | Gender(M/F)  | 23/51          | 11/16          | 0.62 |
|      | Pre-ECT HDRS | -1.15±1.29     | NA             | NA |
|      | Post-ECT HDRS| 16.90±6.70     | NA             | NA |
| Total | Number      | 119            | 61             | NA |
|       | Age          | 55.94±15.87    | 48.56±14.90    | 0.008 |
|       | Gender(M/F)  | 43/76          | 26/34          | 0.99 |
|       | Pre-ECT HDRS | 25.59±6.14     | NA             | NA |
|       | Post-ECT HDRS| 11.48±9.07     | NA             | NA |

Note: M: Male, F: Female, ECT: Electroconvulsive therapy, HDRS: Hamilton depression rating scale DEP: Depression, HC: Healthy control
11.3.3 Comparison of OCR and traveled distance between HC and DEP in pre-ECT and post-ECT

**Figure 11-2A** and **Figure 11-2B** show the OCR values of HC and DEP groups in different states for pre-ECT and post-ECT, respectively. Only OCR of state 2, with relatively higher CCN/DMN functional connectivity, shows a significant difference between DEP and HC.
We found HC spent more time in state2 before applying ECT (FDR corrected \( p = 0.015 \)), while the pattern reversed after ECT, and patients with depression spent more time in state 2 (FDR corrected \( p = 0.03 \)). Moreover, we compared the traveled distance between DEP and HC groups in pre-ECT and post-ECT (Figure 11-2C). The results showed that in pre-ECT, the HC group traveled significantly more distance compared to the DEP group \( (p = 0.04) \). Again, in post-ECT, the HC group's traveled distance is higher than the DEP group, but this difference is not significant.

### 11.3.4 The link between Pre-ECT OCR and the effectiveness of ECT

To predict whether applying ECT would be effective, we correlated the calculated OCR of 119 patients with their associated HDRS change \( (\text{post HDRS-pre HDRS}) \) by controlling the age, gender, scanning site, and the number of treatments. As shown in Figure 11-3, only the OCR of state 1 is a significant predictor \( (R = 0.22, \text{FDR corrected } p = 0.03) \). In more detail, we found those patients who spend more time in state 1, with relatively lower CCN/DMN functional connectivity, showed less reduction in their HDRS. We did not find a significant link between the pre-ECT traveled distance and the HDRS change.
Figure 11-2: A) The OCR comparison between DEP and HC in three distinct states of pre-ECT. A significant difference is observed in state2 (corrected $p=0.015$), B) The OCR comparison between DEP and HC in three distinct states of pre-ECT post-ECT. A significant difference is observed in state 2 (corrected $p=0.03$). In state2, ECT had significantly changed the OCR value of HC and DEP before applying ECT (HC>DEP) compared to after applying ECT (HC<DEP). C) shows the traveled distance between DEP and the HC group in pre-ECT and post-ECT. In pre-ECT, the traveled distance of the HC group is significantly higher than the DEP group ($p=0.04$). After applying ECT, the HC group has higher traveled distance than the DEP group, but this difference is not significant.
In this study, we used rs-fMRI of 119 participants suffering from depression and experiencing a series of ECT treatments and 61 healthy individuals to predict the effectiveness of ECT and investigate brain dynamics in pre-ECT and post-ECT by comparing DEP and HC groups. To this aim, we used dFNC features extracted from CCN and DMN and clustered them into three different states. In this study, using a data-driven method of analyzing dFNC in CCN and DMN, we demonstrated that these brain networks are thoroughly dynamic in both pre- and post-ECT states. This finding agrees with previous studies on MDD that have provided evidence of dynamism in CCN and DMN [339]–[342].

We compared the differences between DEPs and HC groups considering this temporal dynamic activity of the brain. To this aim, we calculated the OCR feature, which shows the proportional amount of time participants spent in each state. Comparing the OCR of DEP with the HC group in pre-ECT, we found that the OCR of the HC group is significantly higher than the DEP group in state 2. This means that the HC group spends

**Figure 11-3: Correlation between OCR values and reported HDRS change (Pre-Post) in three states.** Blue dots are referred to 119 DEP individuals. The bold black line is the fitted curve. R indicates the fitted line slope in each state. As it is shown, state 1 (the state with the lowest CCN/DMN connectivity) significantly predicts the HDRS change based on OCR values, less OCR value corresponds to more HDRS change.

11.4 Discussion

In this study, we used rs-fMRI of 119 participants suffering from depression and experiencing a series of ECT treatments and 61 healthy individuals to predict the effectiveness of ECT and investigate brain dynamics in pre-ECT and post-ECT by comparing DEP and HC groups. To this aim, we used dFNC features extracted from CCN and DMN and clustered them into three different states. In this study, using a data-driven method of analyzing dFNC in CCN and DMN, we demonstrated that these brain networks are thoroughly dynamic in both pre- and post-ECT states. This finding agrees with previous studies on MDD that have provided evidence of dynamism in CCN and DMN [339]–[342].

We compared the differences between DEPs and HC groups considering this temporal dynamic activity of the brain. To this aim, we calculated the OCR feature, which shows the proportional amount of time participants spent in each state. Comparing the OCR of DEP with the HC group in pre-ECT, we found that the OCR of the HC group is significantly higher than the DEP group in state 2. This means that the HC group spends
more time in state 2 than DEPs. The main characteristic of pre-ECT state 2 is having higher CCN/DMN connectivity than other pre-ECT states.

The difference between MDD and HC groups with a specific focus on DMN and CCN has been reported in previous literature. For example, one study reported an increase in within-DMN connectivity for MDD [343], while another study reported a decrease in this network's connectivity [344], [345]. Additionally, recent studies using rs-fMRI suggest an association between depression and abnormal functional connectivity in the CCN network [346]–[351]. Other studies focusing on the CCN network reported attenuated network connectivity in remitted MDDs [352], [353]. Finally, a study tried to predict the antidepressant response in MDDs focusing on within-CCN and within-DMN networks and reported low and high resting functional connectivity within-CCN and within-DMN, respectively [354]. While previous studies focused only on within-DMN and within-CCN functional connectivity, the current study might provide new evidence about the role of CCN/DMN connectivity in depression.

Furthermore, we investigated the effect of ECT on evaluating the temporal dynamic activity of the brain after implementing ECT in post-ECT. We found that the OCR of DEPs is significantly higher than HC participants. After ECT, patients with depression spent more time in state 2 than the HC group. Similar to the pre-ECT state2, the post-ECT state2 shows the highest CCN/DMN connectivity than other states. That might provide new insight into the effect of ECT on the CCN and DMN by regulating the temporal dynamics of these brain networks. Moreover, post-ECT state2 has relatively higher within-CCN connectivity. This contrasts with previous studies that reported reduced within-CCN
connectivity associated with the antidepressant state in a relatively small dataset [354]; we found increased within-CCN connectivity after ECT.

Evaluating the effectiveness of ECT, i.e., identifying patients as potential remitters or non-remitter before implementing ECT, would be valuable from the clinical perspective [355]. On the one hand, many studies have correlated baseline clinical characteristics with MDD status outcomes [356], [357]. Such analyses are based on group-level analysis rather than individual patient-level aspects [358]. There is a need for new metrics to predict ECT outcomes. On the other hand, selecting a feature among many MRI metrics is difficult because they focus on non-overlapping aspects of brain function [359]. Although some metrics are based on functional connectivity of fMRI data, there are focused on static brain region connections [359]. Therefore, the use of metrics based on dFNC and using the correlation analysis of such metrics with behavioral and clinical data could help predict ECT outcomes. In this study, we were able to predict the effectiveness of ECT before applying it. To this aim, we correlated the HDRS change with just pre-ECT OCR of DEPs and found that brain dynamics in state 1 is the predictor.

We found a significant correlation between OCR and HDRS change with a positive slope, which means that the more OCR in pre-ECT state 1, with relatively less CCN/DMN, equals fewer HDRS changes. Therefore, DEPs who spent more time in pre-ECT state1 are less likely to be treated by ECT. Interestingly, the main characteristic of state 1 is that this state has the lowest connectivity between CCN/DMN relative to state 2 and state 3. Also, the results of the effect of ECT showed that ECT had increased the amount of time that DEPs are spending in post-ECT state 2, with higher CCN/DMN connectivity. As such, we can conclude that the results of prediction are in line with the result of the effect of ECT since
spending time in the state with the minimum CCN/DMN correlation is not good, and ECT has increased the amount of time DEPs spend in the state where the CCN/DMN correlation is maximum.

Finally, we extracted the total traveled distance metric from pre-and post-ECT dFNC using Euclidean distance. This metric shows the brain's dynamic since it measures the distance traveled between each window’s dFNC. In pre-ECT, the traveled distance of DEPs is significantly lower than the HC group, but after ECT, this difference is not significant. In other words, ECT decreases the traveled distance difference between HC and DEP. Therefore, we can conclude that ECT makes the brain activity of DEPs more dynamic.

11.4.1 Limitations and future work

There are a few limitations in the current study. First, we did not directly measure whether participants were awake or closed their eyes during the scanning. Concerning this issue, we used the questionnaire and self-reports provided by participants after the scanning finished. Based on the literature, this issue might affect our results [360]. To address this, it is possible to extend the dynamic functional connectivity approach to assess when the participant’s eyes were closed or exhibiting aspects of drowsiness [361]. dFNC approaches have already shown promise in predicting measures of drowsiness or sleep [362].

Moreover, our data were collected in two different sites, and the imaging protocol and the number of treatments were different in these two sites. Addressing this issue, we tried to consider these differences by including site and number of treatments as a covariate in our analysis to control their effect on the results. Finally, although HDRS is generally utilized in scaling the depression symptom severity, this score relies upon the interviewer's skill
and knowledge [305]. Since the data in this study comes from two separate sites, each with its own set of raters. This may cause HDRS values to vary and be inaccurate across sites.

11.4.2 Conclusion

This study evaluated the dynamic functional network connectivity of DMN and CCN using rs-fMRI data of DEP patients experiencing ECT treatment. Focusing on CCN and DMN networks and clustering the brain dFNC to three different states, we found that brain activity in these networks is highly dynamic. Comparing the OCR feature extracted from dFNC of these two networks between DEP and HC groups, we found that the HC group prefers to spend more time in a state where the connectivity between CCN and DMN is the maximum. Moreover, we found that ECT causes an increase in the amount of time DEP patients spend in the state in which the CCN/DMN functional connectivity is maximum. In addition, we could significantly predict the effectiveness of the ECT using just pre-ECT brain activity. We found that the more time participants spend in the state in which the correlation of CCN/DMN is minimum, the less HDRS change they have, and the less effectiveness of ECT they would experience. Finally, we found that the distance that DEP patients travel before ECT is significantly lower than the distance they travel after ECT compared to the HC group. At the same time, this difference was not significant after ECT. This suggests an increase in brain dynamics after implementing ECT. In brief, this study focused on functional connectivity dynamics of CCN and DMN networks of DEP patients and introduced CCN/DMN connectivity as a biomarker by which we can predict the effectiveness of ECT.
CHAPTER 12. CONCLUSIONS AND FUTURE WORK

12.1 Main contributions

This dissertation contributes to the field from both methodological and clinical implications aspects of dynamic functional network connectivity (dFNC). A summary of the contributions is shown in the following paragraphs.

12.1.1 Methodological contributions

1- We developed a new dFNC pipeline to analyze a big dataset of dFNC information. We validated the reliability of the pipeline in four sessions of the Human Connectome Project Young Adult dataset. We showed that our method is 27 times fasters than the conventional pipeline in analyzing dFNC dataset. Our new method can be applied to the other field where we need to cluster a huge amount of data.


2- We developed another dFNC pipeline based on an unsupervised feature learning method to uncover the hidden dynamics of less active regions of the brain. In a larger brain network, a group of brain networks such as visual, sensorimotor, and auditory networks, which are strongly correlated, may mask less-correlated networks and limit spatiotemporal resolution. We introduced a mechanistic approach that can reveal the dynamics of less influential nodes masked by high influential nodes. Our approach can contribute to the characterization of many
neurological disorders and cognitive functions that could lead to the improved
diagnosis and treatment of those disorders.

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3- We developed a new framework based on an explainable machine learning
approach to find the underlying neural process of different neurological and
neuropsychiatric disorders based on FNC information. Although we developed our
framework in the context of static FNC information, this pipeline can be used to
identify the most important features in which we want to differentiate two groups
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4- We introduced a new set of dFNC features that can be used as a potential biomarker
for different brain diseases. These new features can provide new aspects of dFNC
information. Additionally, we developed an open-source MATLAB toolbox to
extract dFNC features.

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12.1.2 Clinical implications of dFNC

12.1.2.1 Alzheimer’s disease

5- For the first time, we analyzed the change in dFNC pattern by AD progression. Our results showed the temporal and spatial pattern of whole-brain FNC differentiates AD from healthy control and suggested substantial disruptions across multiple dynamic states. In more detail, our results indicated that the sensory network is affected more than other brain networks, and the default mode network is one of the last brain networks get affected by AD. In addition, abnormal patterns of whole-brain dFNC were identified in the early stage of AD, and some abnormalities were correlated with the clinical score.


6- For the first time, we explored the link between the genetic risk of AD and dFNC information. We found that higher AD risk reduces within-visual sensory network
(VSN) sFNC and that individuals with higher AD risk spend more time in a state with lower within VSN dFNC. Additionally, we found that AD genetic risk affects whole-brain sFNC and dFNC in women but not in men. In conclusion, we presented novel insights into the links between sFNC, dFNC, and AD genetic risk.


12.1.2.2 Schizophrenia

7- We explored the link between default mode network (DMN) dFNC and symptom severity of schizophrenia. To our knowledge, this was the first study to investigate DMN dFNC and its link to schizophrenia symptom severity. We identified reproducible neural states in a data-driven manner and demonstrated that the strength of connectivity within those states differed between patients with schizophrenia (SZ) and healthy controls (HCs). Additionally, we identified a relationship between SZ symptom severity and the dynamics of DMN functional connectivity. We validated our results across two datasets. These results support
the potential of dFNC for use as a biomarker of schizophrenia and shed new light
on the relationship between schizophrenia and DMN dynamics.

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8- Our work in analyzing VSN dFNC and its link with visual learning deficits in
schizophrenia represents the first focus on functional connectivity dynamics in the
visual system of schizophrenia using resting fMRI and finds substantial disruptions
across multiple dynamic states. Results strongly suggest the need for a renewed
focus on visual sensory network dynamics in schizophrenia both at rest and via
tasks.
12.1.2.3 Major depressive disorder

9- We explored the link between DMN dFNC and symptom severity in major depressive disorder. This study is the first attempt that explores the temporal change in DMN connectivity in a relatively large cohort of patients with MDD. We also introduced a new hypothesis that explains the inconsistency in DMN functional network connectivity (FNC) comparison between MDD and healthy control based on static FNC in the previous literature. Additionally, our findings suggest that anterior cingulate cortex connectivity and the connectivity between the precuneus and posterior cingulate cortex are potential biomarkers for the future intervention of MDD.


10- For the first time, we explored how electroconvulsive therapy (ECT) changes the brain dFNC of a patient with depression. Our finding suggests that dFNC features, estimated from the cognitive control network (CCN) and DMN, show promise as a predictive biomarker of the ECT outcome of patients with depression. Also, this
study identifies a possible underlying mechanism associated with the ECT effect in depression.


12.2 Future work

Although we discussed the future directions associated with the limitations and future work in the Discussion section of each chapter, we discuss some new directions in a broader view.

1- **Real-time dFNC pipeline**: As we showed in Chapter 6 to 11 of this dissertation, dFNC features could be potential diagnosis and response biomarkers in different applications. On the other hand, in recent years, closed-loop forms of intervention such as neurofeedback and closed-loop neuromodulation have shown a potential therapeutic treatment for brain disorders. Therefore, developing a real-time dFNC framework based on iSparse-k-means (from Chapter 2) approach that can analyze streaming rs-fMRI would be very beneficial for using dFNC-based biomarkers as a potential control in closed-loop interventions.

2- **The new version of iSparse k-means**: In Chapter 2, we developed iSparse k-means to analyze big data faster. We highly believe that if we integrate other clustering methods than k-means in our framework, we might get faster results.
Therefore, future work is needed to integrate other clustering methods with the framework proposed in Chapter 2.

3- **Brain state reproducibility:** Despite the significant knowledge that dFNC research has contributed to understanding cognitive processes, fundamental questions remain about whether dFNC states are replicable (i.e., ‘stable’) across multiple rs-fMRI sessions and whether temporal instability may represent a biomarker of disease. Future work is needed to characterize the temporal stability of brain states as a novel biomarker in dFNC and to determine whether brain state instability is associated with symptom severity in different brain disorders.

4- **Analyzing dFNC in individuals being born blind:** Interestingly, a recent study of 467,945 subjects suggested that being born blind may be protective against schizophrenia [363]. Several studies suggested that congenitally blind people adaptively recruit and keep their visual network active for other sensory tasks, for example, high-level language or math tasks, more than healthy people [364], [365]. The study from Chapter 9 showed more functional connectivity in the visual network of healthy people. Our findings highlight the need for further study. It would be interesting, for example, to study the dynamics of the visual system in blind individuals with this approach.
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