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Multichannel Characterization of Brain Activity in Neurological Impairments

Yalda Shahriari
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MULTICHANNEL CHARACTERIZATION OF BRAIN ACTIVITY IN NEUROLOGICAL IMPAIRMENTS

by

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A Dissertation Submitted to the Faculty of Old Dominion University in Partial Fulfillment of the Requirements for the Degree of

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ABSTRACT

MULTICHANNEL CHARACTERIZATION OF BRAIN ACTIVITY IN NEUROLOGICAL IMPAIRMENTS

Yalda Shahriari
Old Dominion University, 2015
Director: Dr. Dean J. Krusienski

Hundreds of millions of people worldwide suffer from various neurological and psychiatric disorders. A better understanding of the underlying neurophysiology and mechanisms for these disorders can lead to improved diagnostic techniques and treatments. The objective of this dissertation is to create a novel characterization of multichannel EEG activity for selected neurological and psychiatric disorders based on available datasets. Specifically, this work provides spatial, spectral, and temporal characterizations of brain activity differences between patients/animal models and healthy controls, with focus on modern techniques that quantify cortical connectivity, which is widely believed to be abnormal in such disorders. Exploring the functional brain networks in these patients can provide a better understanding of the pathophysiology and brain network integrity of the respective disorders. This can allow for the assessment of neural mechanism deficits and possibly lead to developing a model for enhancement in the biology of neural interactions in these patients. This unique electrophysiological information may also contribute to the development of target drugs, novel treatments, and genetic studies. Moreover, the outcomes not only provide potential biomarkers for the diagnosis of respective disorders but also can serve as biofeedback for neurotherapy and also development of more sophisticated BCIs.
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I would like to take this opportunity to thank everyone who supported me in conducting this research. First, I would like to thank my supervisor, Dr. Dean J. Krusienski for sharing his expertise, guidance, invaluable advice and encouragement throughout this process. Without his precious help and dedicated involvement and enthusiasm, this research and the outcomes would never have been accomplished. I would like to thank him very much for supporting me in every step over these past three years.

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Chapter 1

INTRODUCTION

1.1 MOTIVATION AND OBJECTIVES

The human brain is a vastly complex network made up of a large number of neurons, on the order of 100 billion. The electroencephalogram (EEG) is the electrical activity measured from the scalp, that is the result of the coordinated firing of cortical neurons and volume conduction. EEG has been widely used in research and medicine for more than 80 years and can provide clinicians and scientists with unique information related to cerebral dysfunction for individuals with neurological impairments.

Hundreds of millions of people worldwide suffer from various neurological and psychiatric disorders. A neurological disorder refers to any structural, chemical, and/or electrical dysfunction of the peripheral and central nervous system that leads to a range of symptoms such as paralysis, muscle weakness, unconsciousness, etc. Psychiatric disorders are the result of brain dysfunction that cause the patient to feel, think, perceive, and act differently from the accepted social norm.

Over 30 million people around the world suffer from debilitating neuromuscular disorders such as spinal cord injury, cerebral palsy, Muscular Dystrophy (MD), Multiple Sclerosis (MS), brainstem stroke, Amyotrophic Lateral Sclerosis (ALS), etc. In extreme cases, certain neuromuscular disorders such as late-stage ALS can affect the
peripheral nervous system, rendering the individual incapable of producing any voluntary motor activity. This is known as locked-in syndrome (LIS). However, these individuals may still have intact cognitive and sensory capabilities. The inability to reliably communicate with the external world greatly reduces the quality of life and often leads to cognitive decline, among other issues. Thus, the goal of a brain-computer interface (BCI) is to provide a communication channel for these individuals by conveying their intentions from direct measurements of brain activity [3].

Psychiatric disorders such as psychosis, anxiety, and mood disorders also affect many millions of people worldwide. These disorders can also have a stigma attached to them that can greatly impact the quality of life of those afflicted, from social and economic standpoints. Psychotic disorders such as schizophrenia, where patients lose perspective of reality, have gained recent attention due to potentially preventable acts of mass violence by individuals suffering from schizophrenia. While the majority of schizophrenics are nonviolent, a better understanding of these disorders will lead to improved diagnostic methods and treatments that will greatly benefit these individuals and society.

Two unique EEG data sets have been obtained for this research. The first is from nine patients with ALS during a daily BCI task. The patients ranged in age and disease progression as indicated by the ALS functional rating scale (ALSFRS) scores, which show the patients' degree of functional impairment. The data from these patients are compared to thirteen healthy, age-matched controls performing the same task. The second dataset was collected from six Phospholipase C-β1 (PLCβ1)
knockout mice, which are known to have schizophrenic traits, and seven healthy controls, during an ASSR task.

Ultimately, the overriding objective of this dissertation is to create a novel characterization of multichannel EEG activity for these selected neurological and psychiatric disorders based on available datasets. Specifically, this work provides spatial, spectral, and temporal characterizations of brain activity differences between patients/animal models and healthy controls, with focus on modern techniques that quantify cortical connectivity, which is widely believed to be abnormal in such disorders. Exploring the functional brain networks in these patients can provide a better understanding of the pathophysiology and brain network integrity of the respective disorders. This can allow for the assessment of neural mechanism deficits and possibly lead to developing a model for the enhancement of neural interactions in these patients. This unique electrophysiological information may also contribute to the development of target drugs, novel treatments, and genetic studies. Moreover, the outcomes not only provide potential biomarkers for the diagnosis of respective disorders but can also serve as biofeedback for neurotherapy and can lead to the development of more sophisticated BCIs.

1.2 ORGANIZATION AND CONTRIBUTIONS

This thesis is organized as follows. Chapter 2 provides background information about electroencephalography, brain-computer interfaces, and the disorders of interest. The first main focus of this dissertation is the characterization of EEG data collected from patients with ALS. Chapter 3 investigates the most significant EEG
correlates of P300-based BCI performance variations in people with ALS. Using those features, preliminary classification algorithms are implemented and applied, to investigate their potential as P300-performance predictors, for evaluation of BCI readiness in subjects, before performing a BCI task. This chapter provides the first contribution of the dissertation, which is the identification of significant EEG predictors of BCI performance, and, their application to the prediction of successful BCI performance, using different classification algorithms. Chapter 4 uses multivariate autoregressive models (MVAR) in an adaptive direct transfer function (ADTF) method, to identify the brain connectivity patterns in ALS patients during a BCI spelling task, and, to compare the patterns with those obtained from the healthy subjects performing the same task. This chapter provides the second contribution of this dissertation, which is the identification of unique functional connectivity differences between ALS patients and healthy subjects. These new EEG indicators of BCI performance can eventually lead to the development of a more reliable and practical BCI system for ALS patients.

The second main focus of this dissertation is the characterization of brain signals collected from mice models of schizophrenia. Chapter 5 presents the impairment of evoked power responses to an auditory steady state stimulation (ASSS) task in mice models of schizophrenia in comparison to healthy control mice. The characterization of these impairments represents the third main contribution of this dissertation. Chapter 6 provides the last contribution of this dissertation, i.e. a novel characterization of brain functional connectivity and synchrony analysis using the same mouse
models from Chapter 5. The ADTF method is used to identify and compare brain connectivity patterns in healthy and schizophrenic mice models. The synchrony response is also evaluated using phase locking value (PLV), phase lag index (PLI), directed phase lag index (dPLI), and phase locking factor (PLF); and the results obtained using these measurements are compared. The outcomes of Chapters 5 and 6 contribute to better understanding of the neural synchrony and connectivity deficit in mice models of schizophrenia that can provide biomarkers for the diagnosis of the disease. Moreover, the histology of such animal model gives us the possibility of assessing and developing different drug targets for the pathophysiology of such diseases that can eventually be used for human patients.

Chapter 7 concludes the dissertation by discussing and summarizing the main results of the dissertation, as well as proposing future extensions of this work.
Chapter 2

BACKGROUND

2.1 EVOKED RESPONSES IN ELECTROENCEPHALOGRAPHY

The electroencephalogram (EEG) is a measurement of the electrical activity of the brain from the scalp. The spatial resolution of EEG is on the order of centimeters while the temporal resolution is on the order of milliseconds. Figure 1 shows a typical electrode placement of the international 10-20 system. Much of the relevant information in EEG is represented as transient amplitude deflections or changes in the oscillatory power. For general analysis of the EEG spectrum, the spectral power is typically measured over specific frequency bands, which have been respectively linked to changes in attention, sleep activity, cognition, etc. The traditional frequency bands are: delta (<4 Hz), theta (4-7 Hz), alpha (8-15 Hz), beta (16-31 Hz) and gamma (>32 Hz). Because of the attenuation created by the skull and the scalp tissue, the SNR in EEG is too low to yield practical information above 80 Hz, and realistically frequencies are limited to approximately 40 Hz.

Evoked potentials (EPs) are deviations in EEG amplitude as the result of an external sensory stimulus. EPs can be transient or steady-state in nature, depending on the stimulus. Transient responses are typically characterized by one or more amplitude deviations that occur with reference to the stimulus onset. Because of the low signal-to-noise ratio (SNR) of EEG, transient responses are commonly obtained
by averaging over multiple trials. In contrast, steady-state responses are the oscillatory activity in the EEG that results from coordinated, repetitive stimuli. This EEG activity characteristically oscillates at the stimulus frequency, and its harmonics.

Event-related potentials (ERPs) are transient EPs that are not simply innate reflexive responses to a sensory stimuli, but also require a cognitive component such as attention, in order to be evoked. The P300 ERP is a positive deflection in the EEG amplitude that appears in the central and parietal regions 200-700 milliseconds after a rare or novel visual, auditory, or tactile stimulus [4]. This signal is typically elicited using an “oddball” paradigm [5], where the subject attends to the novel or rare target stimuli among the non-target stimuli.
2.2 BRAIN-COMPUTER INTERFACES

A Brain-computer interface (BCI) is a communication system that is not dependent on the brain’s normal output pathways consisting of peripheral nerve and muscles [6]. This system can open a channel for locked in patients to communicate with their external world. In a BCI system, brain signal activity is recorded, processed and decoded, translated into device commands, and finally transmitted to an external device in order to convey the users’ intentions. Therefore, to enhance the performance of a BCI system, research should not only focus on the quality of the brain signal recording, but also improved signal decoding and translation methods.

Figure 2 shows a block diagram of a typical BCI system. The input for the BCI system is the recorded brain signal, which can be electrophysiological such as EEG.
and magnetoencephalography (MEG), or hemodynamic such as positron emission tomography (PET), functional magnetic resonance imaging (fMRI), and functional near-infrared spectroscopy (fNIRS). While these aforementioned modalities are non-invasive, signals can also be acquired invasively. Invasive methods generally have higher signal-to-noise ratio, spectral and spatial resolution, but the associated surgical risk is currently not practical for long-term use. EEG is the most prevalent modality for practical BCIs due to its comparatively low cost and portability. The input is transferred to the signal processing block which performs decoding in the form of preprocessing, feature extraction, selection and translation. Finally, the user is provided with feedback from an application such as a spelling device, a neuroprosthetic, a wheelchair etc. Speed and reliability are two critical factors in BCI, and a myriad of studies have explored new methods for improving the information transfer rate in BCI systems [7, 8, 9, 10, 11].

One of the most extensively researched BCI systems is based on the P300 response, usually in tandem with other ERPs in the EEG. The P300-speller was first introduced by Farwell and Donchin who exploited the properties of the oddball paradigm to enable the user to spell words on a computer by sequentially choosing alphabet letters [12]. In this paradigm, the computer displays a 6 x 6 matrix of characters that flash in a randomized fashion. The user focuses attention on the target character in the matrix and a P300 will be elicited when the character flashes. After multiple trials, the P300 response can be accurately detected and machine learning techniques can be used to identify the target character from the responses
2.3 WADSWORTH CENTER’S BCI HOME SYSTEM FOR PATIENTS WITH ALS

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease caused by motor nervous system impairments that cause muscle weakness and atrophy as a result of loss of action of both upper and lower motor neurons in the brain, spinal cord and brainstem [15]. This causes muscle weakness, fasciculation and atrophy, and ultimately renders the individual incapable of performing any voluntary task. Locked-in syndrome (LIS) can occur in the late stages of ALS and patients with LIS are unable to perform even the most basic voluntary motor tasks, with the exception of possibly retaining the ability to generate slight eye movements. Although patients can suffer from severe dysfunction of the nervous system, other brain functions such as cognition and sensory processing remain intact. These retained abilities without the means of outward communication severely degrade the quality of life for these patients.

BCI systems can be used as a potential method of communication to at least enable these patients to convey their basic needs. Accurately decoding the intention of these patients with the goal of developing an assistive technology remains one of the most challenging topics in the clinical and technical research fields today. Rehabilitation strategies have tried to build a communication way for these patients through developing BCI systems and thus, helped them to convey their intention. Moreover, these strategies have made such patients able to be more involved with
their families and communities. These systems have provided communication for a wide variety of healthy and disabled users, including individuals with LIS [16, 12, 17].

The New York State Department of Health's Wadsworth Center has developed and disseminated a BCI system specifically for severely disabled individuals to use in their homes for basic communication. In particular, the system has undergone extensive evaluation with ALS patients [18]. These patients operate the BCI independently in their own homes seated in a comfortable chair or in their own wheelchair at a distance of 90 cm from the monitor. The users view a matrix containing alphanumeric characters and symbols that flash randomly and are asked to attend to the character they wish to type (see Figure 3).
Figure 3: A subject wearing an electrode cap attends to the 6 X 6 matrix of items displayed on a monitor. Lower left inset: the 6 X 6 matrix and the gray 'text-to-spell' bar showing the word to be spelled ('BROWN') and the target letter for the first trial, the letter 'B', in parentheses at the end of the word. Lower right inset: the 16-channel electrode montage and the standard 8-channel subset (marked with 'X's) [2]
2.4 AUDITORY STEADY-STATE RESPONSES IN MOUSE MODELS OF SCHIZOPHRENIA

Schizophrenia is one of the most chronic, severe, and debilitating neuropsychological illnesses that affects about 1% of the population worldwide. Abnormal sensory experiences, disrupted neural integration, synchronization and connectivity, are common in schizophrenia, ranging from visual and auditory distortions in the normal phase to the chronic hallucinations often reported by schizophrenia patients. Most often schizophrenics exhibit deficits in behavioral measures of tone matching [19], temporal and spatial discrimination, and pitch discrimination [20] in the auditory domain.

It has also been shown that patients with schizophrenia can exhibit EEG abnormalities in event-related potentials and spectral power. One promising approach for investigating the neurophysiological markers of perceptual abnormalities in these patients is the utilization of auditory steady-state responses (ASSRs) of EEG, to assess the network integrity and neural synchrony necessary for accurate sensory processing. ASSRs are elicited by continuous periodic auditory stimuli, which can test the capacity of neural circuits to support oscillatory activity across a range of frequencies. Several EEG and magnetoencephalogram (MEG) studies have suggested that ASSRs are localized to the primary auditory cortex, and can be used to evaluate the subject’s auditory functions and other deficits [21, 22, 23]. The auditory stimuli click train modulation can be amplitude modulated (AM), frequency modulated (FM) or both (MM) [24].
2.4.1 MOUSE MODEL OF SCHIZOPHRENIA

The Phospholipase C-β1 (PLC/β1) knockout mouse model has been suggested as a schizophrenic animal model, which is evidenced by its behavioral endophenotypes homologous to schizophrenia [25]. PLC/β1, a phosphodiesterase that is considered as a rate limiting enzyme, generates inositol 1,4,5-triphosphate (IP3) and diacylglycerol (DAG), from phosphatidylinositol 4,5-bisphosphate (IP2). PLC-β1 plays a crucial role in G protein-coupled signaling pathways in the human cortex [26] and has also been proved to be important in plasticity and post-natal cortical development [27, 28]. A recent microarray study detected decreased transcript levels of PLC-β1, in the dorsolateral prefrontal cortex, in schizophrenia subjects [29]. In addition, PLC-β1 mice exhibit many schizophrenia like phenotypes, including behavioral changes, pharmacological responses, and molecular changes [25, 30].

Acetylcholine functions in the brain using two subtypes of receptors: nicotinic and acetylcholine receptors (nAChRs), which are ligand gated ion channels, and muscarinic acetyl choline receptors (mAChRs), which are G-protein coupled receptors. These mAChRs are known to be involved in high-level cognitive processes, such as selective attention, learning, and memory [31]. mAChRs have been implicated in the regulation of GABA receptor inhibition in the prefrontal cortex [32], thus it is suggested that PLC-β1 may indirectly affect the functioning of GABAergic receptors in PFC [30] which has been involved in schizophrenia and mediates gamma oscillations [33]. It is known that GABAergic transmission plays a vital role in synchronous oscillations and ASSRs, in the gamma band frequency range [34]. Similarly Uhlhaas
et al. (2008) reported that GABA is involved in the generation and synchronization of gamma and beta oscillations [35]. The association of networks of inhibitory interneurons with the neural oscillations in the beta frequency range, has also been shown [36, 37]. Several animal studies have explored the link between GABAergic transmission and its functional role in gamma band neural synchrony, using electrophysiology [38] and novel optogenetics techniques [39].
Chapter 3

AN EXPLORATION OF BCI PERFORMANCE VARIATIONS IN PEOPLE WITH AMYOTROPHIC LATERAL SCLEROSIS USING LONGITUDINAL EEG DATA

3.1 P300-BASED BCI PERFORMANCE VARIATIONS

Significant BCI performance variations have been observed between subjects as well as within subjects, especially patients. Studies that explore BCI performance variations may be categorized into between-subject and within-subject studies. Between-subject studies focus on differences across users, while within-subject studies focus on a single user's performance variations over time. Studies may also rely on data from healthy users or patients with ALS. Patients with ALS have particularly pronounced variations in BCI performance, which can be frustrating and discouraging and may even prevent effective communication. However, most studies that assess performance variations have been performed on healthy subjects that have normal function and do not rely on the BCI for basic communication. Thus, findings from these users may not directly translate to patients.

Some recent efforts have focused strongly on improving BCI performance among users who cannot attain control, for example, a two-choice BCI based on imagined
movement that used 64 electrodes, about an hour period of training, and customized signal processing parameters yielded much better performance and lower illiteracy than the initial study by Guger et al., (2003) [40]. However, these studies still found significant performance variations, with some users unable to attain control. Among P300 BCIs, the new face-speller approach improves performance in healthy users and ALS patients [13, 11]. These and other P300 articles also reported substantial performance variations, an issue which has remained relatively unexplored.

3.1.1 BETWEEN-HEALTHY SUBJECT VARIATIONS

BCI studies with healthy subjects often show that some subjects attain excellent control, others attain marginal to good control, and a minority are unable to attain accuracy high enough for effective communication [40, 41, 42, 43]. For example, in the first study of BCI illiteracy, almost half of 99 participants could not achieve accuracy above 70% in a two-choice BCI based on imagined movement [40]. Later work showed that around 11% of subjects could not achieve accuracies above 80% in the conventional row-column (RC) approach in a P300 BCI [42]. In the third major BCI approach, based on the steady-state visual evoked potential (SSVEP), different studies also found that a minority of users could not attain effective control [42, 43].

Another recent study [44] investigated the differences between BCI-literate and BCI-illiterate groups. Based on data recorded from 52 healthy subjects performing a motor imagery task, the authors found that the BCI literate group had high alpha and low theta power relative to the BCI-illiterate group. The authors proposed that this finding could be used to pre-screen subjects to identify the best BCI approach
for each user. Haider and colleagues showed that, while P3 amplitude elicited by an auditory oddball stimulus was not significantly correlated with P300 BCI accuracy, there was a significant correlation between N2 amplitude and P300-BCI performance over 40 healthy subjects [45].

Recently Haider et al. (2013) investigated the neuroanatomical correlates of BCI performance in 20 healthy subjects, and posited that the structural integrity of white matter structures such as the corpus callosum, cingulum, and the superior-occipital fascicle may predict BCI performance in healthy subjects. This paper also hypothesized that structural characteristics of head size, white matter integrity or cortical surface area are strongly correlated with users' BCI aptitude [45]. Their finding extends prior work [46] showing that white matter architecture influences alpha rhythm dynamics. Different studies investigated alpha rhythm characteristics and their relation to working memory, cognition, and underlying issues in functional neuroanatomy [46, 47]. Klimesch et al. (1999) showed that the alpha frequency band is correlated with cognitive and memory performance, and this power is lower in subjects with different neurological disorders.

3.1.2 BETWEEN-PATIENT SUBJECT VARIATIONS

A few studies have investigated the EEG correlates of between-subject performance variations among patients. One recent study examined the relationship between a wide variety of EEG features and the P300 BCI speller performance in ALS patients [48]. The root-mean-square amplitude, negative peak amplitude at five electrode locations, and power in the theta frequency band over eight electrode locations
were correlated with between-subject performance. However, since each subject participated in a limited number of runs, significant correlations with within-subject performance for these three proposed predictors were not found. A later study investigated the temporal dynamics of attention and their relation to P300 BCI performance in 8 ALS subjects using a rapid serial visual presentation (RSVP) task. The authors hypothesized that the temporal filtering capacity in the RSVP task was a predictor of P300-based BCI accuracy [49]. A newer study with 11 ALS subjects showed a strong relationship between P300 BCI performance and both early positive and negative potentials around 200 ms [50]. They found that the P2 and N2 components exhibit the strongest correlation with BCI performance.

3.1.3 WITHIN-HEALTHY SUBJECT VARIATIONS

Few studies have investigated correlates of performance variations within healthy subjects. A study with fourteen healthy subjects found that attentional activity in the gamma-range is important in the trial to trial performance variations in sensorimotor-rhythm BCIs [51].

3.1.4 WITHIN-PATIENT SUBJECT VARIATIONS

Many BCI users, especially people with ALS, exhibit dramatic variations in BCI performance across or even within BCI usage runs. Users may attain 100% in one run and then perform another run with extremely low performance [52, 53].
3.2 PRESENT DATA

The Wadsworth Center and Helen Hayes Hospital support long-term home use of a P300-based BCI (BCI-24/7) by people with ALS [18]. As mentioned, despite periods of successful usage, home users can experience dramatic variations in BCI performance within and across days [54, 2, 48]. These performance inconsistencies can both frustrate and discourage users and caregivers alike. Home users perform a periodic copy-spelling calibration task. The resulting data are then inspected offline to ascertain system and classifier reliability. To date, these analyses have failed to satisfactorily characterize the variations in BCI performance not accounted for by system reliability.

The aim of this chapter is to identify and evaluate EEG features that significantly correlate with performance on the P300 BCI copy-spelling calibration task. Using those features, preliminary classification algorithms will be applied to test the potential predictors of P300 performance in such patients. Data were collected from nine users with moderate to severe ALS while they operated the BCI independently over weeks and months. Better understanding of the mechanisms of successful BCI use may lead to improved processing methods and thus better and more reliable performance. Furthermore, these insights can lead to methods that help identify suitable candidates for long-term P300 BCI operation, or inform users about their BCI readiness on a given day. Such information may save significant time, effort, and frustration for users and caregivers; and may thereby improve BCI performance and increase satisfaction.
3.3 METHODOLOGY

3.3.1 USERS

The nine male users with ALS were referred for BCI evaluation by the Center for Rehabilitation Technology (CRT) at Helen Hayes Hospital or were self-referred. The users were studied in their homes for a period of 2 to 10 months while operating the BCI-24/7 system independently [18, 2]. Table 1 shows demographic and related information for all users. Users’ ALS revised Functional Rating Scale (ALS-FRS-R) scores ranged between 0 and 32 (median=12, std=12.83) on a 48-pt scale [55], where 48 represents normal function and 0 represents complete loss of function. All studies were approved by the Institutional Review Boards of New York State Department of Health, Old Dominion university, and the VA Central IRB. All users provided informed consent (or assent) for the study.

3.3.2 DATA ACQUISITION

EEG data were recorded using an electrode cap (Electro-Cap International) fitted with eight electrodes (Fz, Cz, P3, Pz, P4, PO7, PO8, Oz). This montage was selected based on prior work exploring optimal montages for P300 BCIs [56]. The EEG was amplified using a g.USBAmp amplifier (g.tec Medical Technologies), digitized at 256 Hz, and band-pass filtered at 0.5-30 Hz. All recording channels were referenced to the right mastoid and grounded to the left mastoid. The electrode impedances were kept below 40 kΩ. All data acquisition, real-time signal processing, and feedback
Table 1: Demographic and performance information for each user. The second column lists the users’ baseline ALS Functional Rating Scale score on a 48-point scale determined prior to the first BCI session. The third column lists the duration of BCI use over which runs were collected. The fourth column lists the total number of runs analyzed and the number of successful runs (accuracy ≥ 70%). The rightmost column lists the performance range of all runs analyzed.

<table>
<thead>
<tr>
<th>User</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
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<tr>
<td>ALS-FRS-R Score</td>
<td>2</td>
<td>32</td>
<td>5</td>
<td>27</td>
<td>0</td>
<td>12</td>
<td>23</td>
<td>7</td>
<td>31</td>
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<tr>
<td>BCI Use (months)</td>
<td>2.5</td>
<td>5</td>
<td>2.5</td>
<td>2.5</td>
<td>5</td>
<td>2</td>
<td>10</td>
<td>9</td>
<td>5</td>
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<tr>
<td>Successful/Total Runs</td>
<td>62/127</td>
<td>48/65</td>
<td>45/52</td>
<td>39/51</td>
<td>34/49</td>
<td>19/29</td>
<td>29/60</td>
<td>49/83</td>
<td>28/36</td>
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<tr>
<td>Performance Range (%)</td>
<td>0-100</td>
<td>10-100</td>
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3.3.3 TASK AND PROCEDURE

Users operated the BCI independently in their own homes over a series of months. They completed a brief copy-spelling/calibration task on a daily or weekly basis [18, 2]. Each instance of this task is known as a run. The average number runs for all users is 61 runs (range 29-127 runs), as listed in Table I. For each run, the user was seated in a comfortable chair or their own wheelchair at a distance of 90 cm from the monitor. The user viewed an 8x9 matrix containing alphanumeric characters and symbols that flashed using the checkerboard paradigm (CBP) [58].

Users were asked to attend to a predetermined sequence of target characters and silently count the number of times each target character flashed. The number of prescribed characters varied between 10 to 20 for each run. Each character in turn was shown in parentheses at the end of the word as it became the target. After 4
sec, the character in parentheses began to flash in groups of 4, 5 or 6 characters with no two items in a group adjacent to each other (i.e., the checkerboard (CB) format [58]). A group flashed every 125 ms (8-Hz flash rate), and the number of flashes varied across users for each selection. After each flash sequence, the EEG was classified by a user-specific classifier as detailed in [56]. Online feedback of the character selected by the classifier was provided directly below the prescribed character.

3.3.4 DATA PROCESSING

The online performance of each run was determined based on the number of correctly selected characters divided by the total number of characters in that run. The spectral and temporal features for each run were computed as described below.

EEG Power Spectra

The power spectrum for each run was calculated using the 256-point Welch's method with a window length of 1 second and 0.5 second overlap, resulting in 1 Hz frequency bins. The average power in five frequency bands was then computed using traditional bands: delta (1-3 Hz), theta (4-7 Hz), alpha (8-14 Hz), low beta (15-18 Hz) and high beta (19-30 Hz). To avoid biases when averaging across all users, each individual spectrum was normalized by dividing by its maximum value. The spectral feature space resulted in a matrix of $N \times 40$ (5 spectral features x 8 channels), where $N$ is the total number of runs for each user.
ERP Components

The target responses were extracted 0-750 ms post-stimulus. The mean of each response was removed and all responses from each run were averaged, resulting in 193 temporal amplitude features for each channel and run per user. To investigate the correlations between characteristic ERP components and online performance, N1 and N2 components were defined as the minimum peaks between 80-170 ms and 220-350 ms respectively, while the P2 and P3 components were defined as the maximum peaks between 190-275 ms and 276-500 ms periods, respectively. To avoid biases when averaging across all users, the ERPs were normalized by dividing by the maximum absolute values for each user. This resulted in a temporal feature matrix of N x 32 (4 temporal components x 8 channels) for each user.

Correlations and Statistical Analysis

Pearson’s correlation coefficient was computed between each temporal and spectral feature and the online accuracy for each channel and user. The correlations corresponding to p ≥ 0.05 were set to zero as not significant, and the significant positive and negative correlations were set to 1 and -1, respectively.

As a more categorical evaluation of performance, runs with actual online accuracies ≥ 70% were labeled as successful and all other runs were labeled as unsuccessful [54, 59]. A two-sample t-test was used to compute the statistically significant differences in the temporal and spectral features for successful verses unsuccessful runs. To increase the statistical power while maintaining an equivalent number of runs for
each user, a fixed number of successful and unsuccessful runs were randomly selected
for each user and combined to compute the p-value. This process was repeated 10
times and the features with an average p-value < 0.05 were designated as significant.

3.3.5 P300-BASED BCI PERFORMANCE PREDICTION

Principal Component Analysis

Principal component analysis (PCA) is an orthogonal linear transformation that
projects the data into a new space in a way that the variables are orthogonal with
each other. The first variable is considered as the first principal component (PC),
and has the highest variance, and the other variables have a decreasing order of
variance respectively. PCA is usually used for dimensionality reduction when the
number of variables exceeds the optimum number of observations needed for a proper
classification. PCA is computed based on the eigenvectors of the covariance matrix
as given below:

\[ X^T X \alpha = \lambda \alpha \]  \hspace{1cm} (1)

where \( X \) is the data matrix and \( \alpha \) and \( \lambda \) are the eigenvectors and eigenvalues of
the covariance matrix of \( X \) respectively. Based on the required optimum number of
variables with the highest percentage of variance, a spatial weight \( W \) is formed and
multiplied by the data matrix. Thus, it is projected into an orthogonal space with a
lower dimension as shown below:
\[ Y = WX \] (2)

**Linear Discriminant Analysis**

Linear discriminant analysis (LDA) is a version of Fisher linear discriminant (FLD) analysis and is built based on the linear relationship between a set of training data examples and the corresponding class labels with the goal of maximizing the distance between datasets from different group classes. The weights (W) are obtained as shown below, based on the auto correlation and cross correlation values:

\[ W = R^{-1}C = (X^T X)^{-1}X^T T \] (3)

where C is the cross correlation and R is the auto correlation matrix. X is the training dataset and T is the corresponding class label. The model is built based on a linear relationship between input and output as below:

\[ y = \sum_{i=0}^{D} W(i)X(i) \] (4)

Where W is the weight and X is training data set or regressor and D is the number of inputs.

**Stepwise Linear Discriminant Analysis**

Stepwise linear discriminant analysis (SWLDA) is an extended version of Fisher linear discriminant (FLD) analysis. This method not only performs classification,
but also performs feature selection based on a combination of forward and backward stepwise analyses, and selects the features with the lowest p-value and removes those with p-values greater than 0.15, this is continued until the maximum number of features, which has been set to 11 in this study, are obtained [4, 60]. Using this method, a multi linear regression of the inputs is performed, which does feature selection as well as classification.

**Multilayer Perceptron**

Multilayer perceptron (MLP) is a feed forward neural network which tries to map the output based on a combination of weights and inputs through an activation function as below:

\[
y = W \times \phi \left( \sum_{i=0}^{D} W_2(i)X(i) \right) + W_3 \times X
\]  

(5)

where \( \phi \) is a sigmoid activation function in this study, and \( W, W_2 \) and \( W_3 \) are the weights. Neural networks have been extensively applied and show promising results in the BCI and EEG classification applications [61, 62]. Figure 4 shows a typical form of a three layer MLP neural network.

Based on the output-weights-optimization-back propagation (OWO-BP) algorithm and the least square minimization error, the weights are updated and, therefore, the neural network is being trained. In this chapter a three layer MLP neural network (input-one hidden layer-output) has been evaluated for P300 performance prediction.
Support Vector Machine

Support vector machine (SVM) is another supervised learning method that is trained in a way to construct a hyper plane which maximizes the distance between data belonging to different class labels [63]. With respect to the training datasets and the corresponding class labels, the weights are trained, and they are then evaluated based on the test datasets. The class labels in the test datasets are determined based on the projection of \( X \) on the weights \( W \) with the decision hyper plane as below:

\[
W \cdot K(x) + b = 0
\]  

(6)

where \( W \) are the weights obtained in training phase and \( K(x) \) is the kernel function. In this study two types of kernel functions, namely the linear and the quadratic,
were used for evaluation of linear and non-linear kernel functions. The class labels are assigned to be \( y_i = \{-1, 1\} \) and the constraints \( y_i(w.k(x_i) - b) \geq 1 \) \((i=1,...,n)\) should be satisfied so that they result in the margin \( \gamma = 2/||w||^2 \). Maximizing the margin yields in minimizing \( ||w||^2/2 \) which can cause an optimization problem that can be solved using Lagrange multipliers \( \alpha_i \) as below:

\[
\arg\min_{(a \geq 0)} \left\{ \frac{1}{2}||w||^2 - \sum_{i=1}^{n} \alpha_i (y_i(w.x_i - b) - 1) \right\}
\]

(7)

where \( \sum \alpha_i y_i = 0 \) and \( n \) is the number of training observations. The weights resulted from optimization problem solution can be obtained as below:

\[
w = \sum_{i}^{n} y_i \alpha_i x_i
\]

(8)

10-fold cross validation was used to evaluate the performance of each of the four classification algorithms in the respective P300 performance prediction. Two different types of temporal and spectral features were used for each classifier. For the temporal and spectral domain respectively, four different ERP components of N1, N2, P2 and P3 and five different frequency bands of delta, theta, alpha, low beta and high beta were used. To avoid biasing in the prediction outcomes, in each fold, the number of successful and unsuccessful runs were selected equally, based on the minimum number available. PCA was applied for each feature group separately and the PCs corresponding to the 99% variance were selected for the classification. Four different types of classifiers, i.e. LDA, SWLDA, MLP and SVM with linear and quadratic
kernel functions, were used for prediction.

3.4 RESULTS

3.4.1 PERFORMANCE VARIATIONS

Figure 5 presents performance variations across different days for four representative users. The y-axis reflects the classification accuracy and the x-axis shows the number of days after the initial BCI session. The vertical bars for a single day indicate the range of performance variation over all copy-spelling runs for a single day, from minimum to maximum performance. Notably, some days exhibit dramatic performance variations, while other days show little or no variation. Furthermore, daily range of performance differences can also vary drastically from day to day.

3.4.2 PERFORMANCE CORRELATIONS WITH FREQUENCY BANDS

Figure 6 shows topographies of the thresholded correlation values (i.e., ±1) averaged across users. Eight of nine had significant positive correlations between the alpha band and performance at channel PO8, with an average correlation coefficient of 0.45. Seven of the users showed alpha band significance at channels Cz, P3, Pz and Oz, with average correlation coefficients of 0.37, 0.39, 0.41 and 0.41, respectively.

Delta-band activity was negatively correlated with performance over channel Oz in eight of nine users with an average negative correlation coefficient of -0.42. Seven of nine users also showed significant delta-band correlation at channels PO7 and PO8 with average negative correlation coefficients of -0.41 and -0.44, respectively.
Figure 5: Performance variations for four representative users. The y-axis presents classification performance (as % accuracy), and the x-axis presents the number of days after the initial session. Each connected data point indicates the mean accuracy for the day. The vertical bars reflect the minimum and maximum performance for that day. Data were not available for the days without vertical bars.

3.4.3 PERFORMANCE CORRELATIONS WITH TEMPORAL ERP COMPONENTS

Figure 7 shows the thresholded correlation between performance and the 4 temporal ERP components. Seven of the users had a significant positive correlation between P2 maximum peak amplitude over channels Fz and Cz with average correlation coefficients of 0.44 and 0.46, respectively. Consistent significant correlations between the N1, N2, or P3 components and performance were generally not observed
Figure 6: Average topographies of significant correlations between online accuracy and spectral power. The correlations corresponding to $p \geq 0.05$ were set to zero as not significant, and the significant positive and negative correlations were set to 1 and -1, respectively.

Figure 7: Average topographies of significant correlations between online accuracy and N100 and N200 minimum peak amplitude (two left panels) and P200 and P300 maximum peak amplitude (two right panels). The correlations corresponding to $p \geq 0.05$ were set to zero as not significant, and the significant positive and negative correlations were set to 1 and -1, respectively.

3.4.4 WAVEFORMS AND SPECTRA FOR SUCCESSFUL AND UNSUCCESSFUL RUNS

Figure 8 shows the spectral power amplitude averaged over all users for each channel. The blue and red colors indicate the averages for the successful and unsuccessful runs, respectively. The solid bars indicate the statistically significant differences for each frequency. Higher power is observed in the delta band for unsuccessful runs.
compared to the successful runs for most channels. In contrast, lower power is observed in the alpha and beta frequency bands for most channels for the unsuccessful runs relative to successful runs.

Figure 9 shows the average target ERPs for successful and unsuccessful runs in blue and red, respectively. The solid bars indicate the statistically significant differences for each time point. These results show a distinct positive peak around 220 ms in most channels (median=220.66, std=7.84 at Fz and median=223.63, std=6.79 at Cz), with higher amplitude in successful runs compared to unsuccessful runs. There is also a significant negative peak around 550 ms in Cz, Pz, P3, and P4 that has a higher amplitude for successful runs compared to unsuccessful runs.

3.4.5 CLASSIFICATION RESULTS

Figure 10 shows the mean and standard deviation of classification results for all types of classifiers, using the two different feature types. The classification metrics reported are the sensitivity, the specificity and the accuracy, to give insight about potential class imbalance biases. For the temporal features, the MLP classifier had the maximum performance with accuracy, sensitivity and specificity having means of $66.42\% \pm 19\%$, $65.77\% \pm 20\%$ and $66.76\% \pm 9\%$, respectively. The quadratic SVM had the lowest performance with accuracy, sensitivity and specificity having means of $52.61\% \pm 5\%$, $52.63\% \pm 8\%$ and $52.6\% \pm 6\%$, respectively. For the spectral features, the linear SVM had the highest performance with means of $66.42\% \pm 10\%$, $61.14\% \pm 15\%$ and $71.69\% \pm 12\%$, respectively. The quadratic SVM had the lowest performance using temporal features, with means of $56.68\% \pm 7\%$, $51.67\% \pm 11\%$,
and $61.69\% \pm 9\%$, respectively.

3.5 DISCUSSION

P300 BCI users, especially persons with ALS, can exhibit substantial performance variations within and across usage sessions. In eight of nine users, alpha band activity was significantly correlated with performance over midline sites (Cz, Pz, Oz), P3, and especially PO8. Seven users also showed negative correlations between delta band activity and performance over Oz, PO7, and PO8. Across frequency bands,
increased alpha-band power and decreased delta-band power generally reflected improved performance. Successful runs were also positively correlated with normalized ERP activity around 230 ms in most users over several channels. This was also apparent in the notable positive correlation between P2 amplitude and performance in seven out of nine of users.

These results extend prior work that has explored how EEG oscillations and phasic ERP changes are related to attention, memory, and cognitive demands. Higher alpha-band activity, and lower activity in other bands, may indicate that users are
more attentive and thus more likely to generate robust responses with changes in spectral amplitude reflecting attention [64, 65]. Riccio et al. (2013) also argued that P300 BCI performance is correlated with attentiveness, concluding that “the ability to keep the attentional filter active during the selection of a target influences performance in BCI control” [66]. Another possible explanation of the increased alpha and decreased delta activity during successful sessions may be the association of changes in these bands with cognitive processing [67, 68, 69, 70]. Increased delta activity and decreased alpha activity are also linked to drowsiness [70], which would likely adversely affect performance. However, our results do not show the increase in theta activity that is also a key indicator of drowsiness. Other work has suggested that more detail is necessary to thoroughly characterize such relationships. For example, generation of different ERPs and phasic activities likely reflects subcomponents of semi-independent processes [71, 72]. Indeed, ERP generation seems to entail phase resetting, which is difficult to assess in heavily averaged data. New or revised methods to explore such brain activities in more detail could provide further insight regarding underlying processes and how BCIs might be better adapted accordingly.

The temporal ERP results also extend related prior BCI studies. For example, Halder et al. (2013a) found that P300 amplitude in a P300 BCI context was correlated with P2 and N2 activity, as well as delta activity in an auditory oddball task for participants with ALS [50]. Related work in healthy participants using an auditory oddball task also found the interesting result that P300 amplitude was not strongly correlated with visual P300 BCI performance, whereas activity around 200 ms and
later negative activity (in their case, from 400-600 ms) was predictive of performance [45]. That study also found that P300 BCI performance could be predicted fairly well by the auditory oddball task prior to each session. The correlation of activity around 200 ms is consistent with the findings of the present study in participants with ALS performing a visual P300 task.

Our results showed strong performance variations, both within and across sessions. The literature provides mixed results from long-term use of P300 BCIs by people with ALS. Sellers and Donchin (2006) found that there were substantial variations in relevant EEG signals, but that these did not strongly affect performance [54]. Nijboer et al. (2008) reported that variations in key EEG components and performance were relatively minor across 40 weeks of BCI use by patients with advanced ALS [73]. McCane et al. (2014) and Silvoni et al. (2013) also found modest performance variations across sessions with ALS patients, which were not correlated with disease progression [2, 74]. Recently, Sellers et al. (2014) found that a long-term user with brainstem stroke could use a P300 BCI for effective communication in 40 of 62 sessions over 13 months [75]. Such performance variations may reflect changes in motivation, implying that assessment of user's motivation - and methods to motivate users - could improve performance [76, 77].

Our current analyses of spectral and ERP activity help elucidate cognitive and neural function during a focused attention task in ALS patients. These analyses, in tandem with other studies, may also encourage developments that improve performance during real-time BCI use or offline adaptation. These include new signal
Figure 10: Classification performances (accuracy, sensitivity and specificity) for all types of classifiers using (a) temporal and (b) spectral features.
processing and classification methods, specific electrode montages, methods to improve motivation, analysis approaches based on single-trial dynamics, and changes to display and task parameters (e.g., highlight specific ERP components). This could also improve prediction of P300 BCI performance both within and across sessions, which could help users decide whether to start, continue, postpone, or end a BCI usage session. The methods and materials in this study is easily transferable to other real-world BCI research and usage activities. Data is collected from ALS patients using P300 BCIs in a field setting, using a portable amplifier and a fairly small electrode montage, with sites typical of P300 BCIs [2, 18].

Using several simple machine learning algorithms, the preliminary results for P300 performance prediction showed that the MLP and the linear SVM can serve as potential predictors of P300 speller readiness for ALS patients. In fact, the preliminary results indicated that using both spectral and temporal features, a prediction performance (i.e. accuracy = % 66) well above chance accuracy (i.e. chance accuracy = % 50) can be obtained. The methodology in this study is easily translatable to other real-world BCI research and practical activities.

Overall, this chapter mainly explored the correlations between BCI performance and the post-processed power spectra and ERP components. The results could lead to the development of new methods to improve P300 BCI performance, identify the best BCI parameters for each user, predict and adapt to performance variations, and which can lead to a better understanding of brain function in people with ALS.
Chapter 4

DIRECTIONAL BRAIN FUNCTIONAL INTERACTION ANALYSIS IN PEOPLE WITH AMYOTROPHIC LATERAL SCLEROSIS

Exploring the functional brain networks in ALS patients can provide a better understanding of the neuropathology and possibly lead to more effective interventions. Most recent studies with ALS patients have investigated the functional connectivity between different brain regions during specific cognitive tasks and resting state [78, 79, 80]. However, few existing studies characterize the effect of ALS on brain network connectivity [79, 81].

A study by Mohammadi et al. using ICA based fMRI analysis in a resting mode found a decreased connectivity in the default-mode network as well as the sensorimotor network for ALS subjects compared to healthy controls [80]. A later study by Douaud et al. using resting state fMRI data from ALS subjects found a distinct pattern of connectivity spanning sensorimotor, premotor, prefrontal and thalamic regions in the ALS patients compared to healthy controls [79]. Another recent study by Blain-Moraes et al. using normalized symbolic transfer entropy during a cognitive spelling task found a significantly higher connectivity in the parietal to frontal feedforward connections in ALS patients compared to healthy controls. However, this study did not show a significant difference between feedback connectivity between these two groups [78].
The aim of this chapter is to investigate brain connectivity differences associated with performance variation in ALS patients. This is accomplished by evaluating the causal relationship of scalp-EEG recording during the execution of the P300 speller task by people with ALS as compared to healthy controls. This analysis can provide insight to the neurophysiological differences that impact task performance and may lead to improved processing techniques for future BCIs.

4.1 METHODOLOGY

4.1.1 ADAPTIVE DIRECTED TRANSFER FUNCTION

Directed transfer function (DTF) analysis is a method which measures the causal relationship between two or more signals and can be considered the extension of the bivariate connectivity measuring methods. Adaptive directed transfer function (ADFT) is the extended version of DTF that has the advantage of measuring the rapid changes of the connectivity relationships between different brain regions. Specifically, it can be used for non-stationary signals and the signals with short duration such as event related potentials (ERPs)[82, 83, 84]. The method takes into account the influence of all the other signals on a specific signal in the region of interest based on adaptive multivariate autoregressive (AMVAR) modeling. Equation 9 shows an AMVAR model where \( y(k) \) is a d-dimentional vector at time point \( k \), \( y_d \) is the \( d^{th} \) dimension of \( y \), \( \varepsilon \) is a Gaussian white noise and \( A(r,k) \) (i,j) is the d x d dimension time varying model parameters or AMVAR model coefficients and can measure the influences from variable i to j after r time points at time point k.
where \( p \) is the AMVAR model order which can be optimally determined based on Final Prediction Error (FPE) criterion [85] and Schwarz Bayesian Criterion (SBC) [86]. The coefficient matrix \( \textbf{A} \), can be estimated based on Kalman filter algorithm [87]. By getting the Fourier transform of the estimated time varying AMVAR coefficients \( \textbf{A} \), we can obtain \( \textbf{A}_k(f) \), which shows the AMVAR coefficients at both time point \( k \) and frequency \( f \) as below:

\[
\textbf{A}_k(f) = \sum_{r=1}^{p} \textbf{A}_{(r,k)} e^{-i2\pi f r}
\]

We can write the transfer function of the multivariate regression model in equation 9 as below:

\[
H_k(f) = [I - \textbf{A}_k(f)]^{-1}
\]
ADFT values can be determined as below [83]:

\[ \gamma_{ij}^2 (f, k) = \frac{|H_{(k,i,j)}(f)|^2}{\sum_{m=1}^{H} |H_{(k,i,m)}(f)|^2} \]  

(12)

where \( H \) is the number of channels.

### 4.1.2 CONNECTIVITY STATISTICAL ANALYSIS

The resulting ADTF values cannot be evaluated until a significance test is performed to compare the resulting connectivity values to the values obtained from surrogate data. The method for generating the surrogate data used for the present analysis was based on phase shuffling, which preserves the power spectrum of the signal as well as the linear correlation between the time series. To do this, the FFT of the original signal was obtained and the resulting phase angles were shuffled using a random permutation of the order. An inverse FFT was used to convert the phase-shuffled signal back to the time domain. This shuffling process is repeated many times and the significant connectivity is computed based on the distributions generated by the surrogate data.

### 4.1.3 DATA PROCESSING

All runs for the healthy controls were successful using this criterion. Six EEG channels that are standard for the P300 speller were used to assess brain connectivity: Fz, Cz, Pz, PO7, PO8, and Oz. Average target ERPs segmented from 0-800 ms were used for the connectivity analysis. For consistency, the first 360 trials related to the
target ERPs for both successful and unsuccessful runs of ALS subjects as well as the controls were included in the analysis. For each trial, baseline correction was performed by removing the mean of each response. Significant ADTF values were obtained using eConnectome software [83] with the maximum model order set to 5. The statistical test was performed using 200 surrogate samples with a significance level of $\alpha = 0.05$. The significant and the non-significant ADTF values were set to 1 and 0, respectively, to show the percentage of subjects with significant connectivity values for each connection and time point. To investigate the connectivity pattern in different frequency bands, the values were averaged over the frequency bands of interest; delta (1-3 Hz), theta (4-7 Hz), alpha (8-14 Hz), low beta (15-18 Hz) and high beta (19-30 Hz).

4.2 RESULTS

For the healthy controls, there was mild significant connectivity outside of the high-beta band. For the ALS patients, the significant connectivity was similarly found in the high-beta band, but there were also more broadly distributed connectivity patterns in the other frequency bands compared to the healthy controls. Thus, the analysis is focused on the high-beta band for both subject groups. Figures 11 and 12 show the percentages of subjects in each group with significant frequency band of interest ADFT values for all the channel pairs and time points. For the ALS subjects, the connectivity analysis was computed on the successful and unsuccessful runs separately. Figure 13 shows the percentage of signal outflow to inflow information rate for high beta band at $t=600$ ms. This time point represent the location of the
highest percentage of subjects with significant ADTF values across subject groups.

This figure shows the information flow graphics of connectivity using directional arrows from out to in. These arrows show the causal connectivity from one channel to the other channel (out to in). The color and width of arrows correspond to the percentage of subjects who had significant information flow rate from one channel to the other channel at that specific time point over the high beta frequency band. Only the arrows in the range of 50% to 100% of the maximum percentage of the subjects with significant directional connectivity (out to in) values have been displayed in this figure. It is observed that in the high beta frequency range and time point around 600 ms after stimulus, 80% of the control group had significant information flow from Fz to Cz. Meanwhile, 70% and 60% of the ALS subjects had significant directional connectivity from Fz to Cz in the successful and unsuccessful runs respectively. 80% of ALS subjects had significant DTF values from Po7 to Pz while just near 50% of them had a significant connectivity in this direction over the unsuccessful runs. Pz had the highest percentage of subjects with significant connectivity information inflow rate for all the groups.

4.3 DISCUSSION

There was higher percentage of subjects with significant connectivity in both subject groups in the high-beta band compared with the other frequency bands. These findings can possibly be explained by the association of networks of inhibitory inter-neurons with the neural oscillations in the beta frequency range [36]. The results also indicate a generally broader distribution of significant connections for the
Figure 11: The percentage of significant DTF values for the delta, theta, and alpha bands for the ALS and control groups.
Figure 12: The percentage of significant DTF values for the low and high beta bands for the ALS and control groups.

Figure 13: The percentage of signal outflow to inflow information rate for the high-beta band at t = 600 ms. Left: controls (all successful), Middle: successful ALS runs, Right: unsuccessful ALS runs.
ALS patients compared with the control group. This corresponds with the findings obtained by Douaud et al. (2011) [79], which attributed the increased brain connectivity in ALS patients to a physiological, compensatory response to disease-related loss of structural network integrity. However, this broader distribution may also be partially attributed to higher variability in the connectivity patterns for the ALS group.

It is interesting to note that the channel pairs and approximate time instances with the highest percentage of significant beta-band connectivity across subjects are consistent for the successful ALS runs and the control group, which consists of all successful runs. This may be associated with task engagement for the successful runs since this pattern appears sufficiently suppressed for unsuccessful ALS runs. Also, it is interesting to note that the highest percentage of peak activity is observed around 600 ms at the bidirectional connection of Fz-Cz for the ALS and control groups. While the peak of the P300 is generally later for ALS patients, this connectivity analysis may reflect common connectivity patterns in the later stages of the P300 response across groups. The directivity analysis shows that there are few, very distinct connections that only exist between adjacent electrode pairs for the control group. The information flow is primarily outward from Cz and Oz, with PO7 flowing to Pz and Oz. The highest percentage of flow is from Fz to Cz. In contrast, there are complex information flow patterns for both the successful and unsuccessful runs of the ALS patients. This also supports the findings in Douaud et al. (2011) [79] but does not clearly discriminate the performance groupings.
The purpose of this study was to determine if a comparison of measures of causality derived from EEG data recorded from people with and without ALS while using a P300-based BCI could shed light on day-to-day variations observed during home use by people with ALS. These preliminary findings suggest that there may be differences in brain activity between individuals with and without ALS and, more importantly, between successful and unsuccessful sessions. As expected, the number of significant connections was lower for the unsuccessful ALS group as compared to the successful ALS group and the control group. Consistent with prior research, both of the ALS groups (successful and unsuccessful) exhibited more overall connectivity than the control group, although it remains unclear whether this is a result of inter-subject variability or more expansive connectivity in ALS subjects. Based on these results, criteria can be established to provide home users with information about their readiness to use a BCI and ultimately, it may lead to improved methods to assess and improve BCI performance for all BCI users.
Chapter 5

IMPAIRED EVOKED AND INDUCED POWER RESPONSES TO AUDITORY STEADY STATE STIMULATION IN MOUSE MODELS OF SCHIZOPHRENIA

Typical ASSR characteristics of individuals with schizophrenia are deficits in auditory steady-state entrainment, reduced power in the gamma range (40 Hz) stimulation, and delayed phase synchronization and desynchronization to click trains [88]. A study by Vierling et al. (2008) showed reduced 40 Hz responses and increased 20 Hz responses to auditory steady-state stimulation (ASSS) [89]. Recently, Vohs et al. (2012) investigated the effects of GABA agonist and NMDA antagonist on the ASSR of a rat model with neonatal ventral hippocampal lesions (NVHLs) as a neurodevelopmental model of schizophrenia in five ASSR stimulating frequencies of 10, 20, 30, 40 and 50 Hz [90]. They found that both ketamine (NMDA antagonist) and muscimol (GABA agonist) increase the phase locking to the stimulating click train as well as the power spectrum in the SHAM rats model compared with the NVHL ones mostly in the frequencies of 20-40 Hz.

The aim of this chapter is to characterize the impaired temporal and spectral features in PLC-β1 mice during an auditory steady state stimulation (ASSS) task and compare them with healthy controls. The event-related power spectral perturbation
(ERSP), raw baseline power and auditory evoked potential (AEP) at each electrode location are examined.

5.1 METHODOLOGY

5.1.1 ANIMAL PREPARATION AND ELECTRODE IMPLANTATION SURGERY

F1 homozygous mice and wild-type littermates were obtained by crossing C57BL/6J(N8) PLCβ1+/- and 129S4/SvJae (N8) PLCβ1+/- mice. PLCβ1 mice belong to this genetic background and were free from complications such as severe seizures, undeveloped growth and early death that were observed in the original background [91]. More specific details such as generation of animal and genotyping method using polymerase chain reaction (PCR) analysis were described in the work of Kim et al. (1997) [92]. All the experiments performed on 10-14 week old male mice which are of approximately 24g body weight. Seven wild type mice and six PLC-β1 mice were used in this study.

For implantation of electrodes on frontal and auditory cortex, animals were anesthetized by intraperitoneal dose of ketamine/xylazine mixture (120 and 6 mg/kg, respectively) and then head-fixed on a stereotaxic apparatus (David Kopf Instruments, CA, USA). After shaving the hair, an incision was made to expose the skull by holding the skin with micro clamps. Before electrode implantation the skull was cleaned using saline to avoid dryness and to remove extra membrane debris. Two microscrew-type electrodes (chrome-plated stainless steel, 3 mm in length and 1 mm
in diameter, impedance of 20 k, Asia Bolt, Seoul, Korea) were bilaterally implanted on the frontal cortex (1.10 mm anterior and 1.75 mm lateral to bregma) and two wire-type electrodes (Teflon-insulated tungsten wire, 76.2/114.3 m in bare/coated diameter, A-M Systems, Sequim, WA, USA) were bilaterally implanted in auditory cortex (2.92 mm posterior, 3.75 mm lateral and 1.2 mm ventral to bregma). For ground and reference, two screw electrodes were implanted on the cerebellum. After implantation of electrodes, glue was applied around the electrodes on the skull to prevent disconnection. Dental cement was carefully coated over the electrodes to fix them on to the skull. After coating, the skin was sutured and an antibiotic cream was applied to avoid skin infections. The animal was placed back into the cage and allowed to recover for one week. After experiments, histology was performed to verify the placement of electrode in the auditory cortex.

The surgical, handling, and experimental procedures for all EEG recordings and anesthesia were conducted in accordance with guidelines by the Institutional Animal Care and Use Committee in Korea Institute of Science and Technology. All the mice used in this study were treated according to the Act 1992 of the Korea Lab Animal Care Regulations and Associated Guidelines.

5.1.2 ASSR STIMULATION PROCEDURE

During the recording, the mice were presented with auditory steady-state stimulations in the form of trains of clicks. Stimulus presentation was performed using LabVIEW software (National Instruments, Austin, TX, USA). These clicks were presented through speakers placed in a closed recording chamber. The stimuli were
presented as trains of clicks which varied in frequency (20, 30, 40 and 50 Hz). The duration of each click was 500 ms and inter-train interval was 700 milliseconds [88], and 1800 trials were conducted for each stimulus frequency.

5.1.3 EEG RECORDING

Two channels of electroencephalogram (EEG) and two channels of local field potential (LFP) electrodes were implanted in frontal and auditory regions, respectively. Recordings were commenced after one week of recovery from surgery. Electrophysiological recordings were performed continuously from mice under freely moving condition. Recordings included 15 mins of baseline, 2:40 mins of stimulation period and 15 mins of recovery baseline, which was recorded after the stimulation period, has stopped. Prior to recording, each mouse was habituated under freely moving state in a recording chamber for 3 hours. All the signals were acquired by a Synampe2 amplifier (Neuroscan Inc., El Paso, TX, USA) and digitized with an analog-digital converter (Digidata 1440A, Molecular Devices, Sunnyvale, CA, USA) at a sampling frequency of 500Hz. Figure14 shows the electrode placement and experimental setup for an ASSS task.

5.1.4 HISTOLOGY

Following the completion the recording, the mice were sacrificed (under 2% Avertin anaesthesia) exsanguinated with saline, and perfused transcardially with a solution of 10% phosphate buffered solution. Brains were post-fixed for three days, and then transferred to a 30% sucrose solution for 48 hours at 4 degrees C. Brain tissue was
Figure 14: Experimental setup for ASSS stimulation. (A) Two channels of electroencephalogram (EEG) and two channels of local field potential (LFP) electrodes were implanted in frontal and auditory regions, respectively. FC, frontal cortex; AU1, primary auditory cortex; R, reference; G, ground; B, bregma; L, lambda. (B) Time profile of EEG recording during ASSS. 15 mins of baseline before stimulation and 2 hrs 40 mins auditory stimulation and 15 mins of recovery baseline after stimulation. (C) Stimulation period was 1 millisecond duration click which is divided into on and off states.
cut at a thickness of 40 m on a freezing microtome and collected into wells contained PBS. Tissue was washed with saline 2-3 times and mounted onto slides, dried, and coverslipped using Vectashield hard set mounting medium along with DAPI staining. Images were taken using a Fluorescence microscope.

5.1.5 DATA ANALYSIS

The signals were preprocessed and analyzed using MATLAB (MathWorks, Natick, MA, USA). Since each electrode can have a different contact impedance, the EEG time series amplitudes corresponding to each electrode were first normalized. The resulting EEG signals were segmented into epochs containing click trains, 0.5 second pre-stimulus period and 0.5 second post-stimulus period and band pass filtered (5 – 55 Hz) using a 6th order Butterworth filter. After baseline correction, EEG signal epochs that were contaminated with noise and muscle activity artifacts were excluded. This was performed by using a subject-specific threshold of the standard deviation for the 100-Hz high-pass filtered signal.

Auditory Evoked Potentials (AEPS)

The auditory evoked potentials (AEPs) in response to the stimulus were averaged over the two auditory and frontal channels, respectively, for each group. The P1 and N1 amplitudes and latencies were measured and compared between each across each groups. N1 was defined as the minimum peak within 20-70 ms post-stimulus and P2 was defined as the maximum peak within 50-140 ms post-stimulus.
ASSR spectral analysis

The EEG data were bandpass filtered from 15 - 55 Hz using a zero-phase FIR filter. A short-time Fourier transform was used to evaluate the temporal dynamics of the power spectrum using window length of 240 ms and 200 ms overlap at a frequency resolution of 1 Hz. Event-related power spectral perturbation (ERSP) was obtained by subtracting the mean power of each trial from its baseline period and averaging over all trials.

5.2 RESULTS

5.2.1 PEAK AND LATENCIES OF AUDITORY EVOKED POTENTIALS (AEPS)

Figure 15 shows the average AEPs to the 40 Hz stimulus from 100 ms pre-stimulus to 600 ms post-stimulus for each group. AEPs appeared as N1-P2 complex in auditory cortex, whereas P2 was not obvious in frontal cortex in several mice. An obvious morphological difference was observed between two groups at both auditory and frontal cortices. The PLC-β1 null group exhibited a delayed and reduced N1 component over the auditory cortex compared to the wildtype group. 7 out of 8 mutant mice showed no clear evidence of an evoked response in the frontal cortex. All the values below are mean ± SEM.

N1 Component

Amplitudes: The wildtype group exhibited an N1 amplitude of \(-0.17 \pm 0.04 \mu V\)
and 0.33 ± 0.08 μV for the frontal and auditory channels, respectively. The PLC-β1 null group exhibited an N1 amplitude of 0.24 ± 0.03 μV for auditory channels with no consistent AEP found for the frontal channels. Although there was a significant reduction in the N1 amplitude of mutants over the frontal cortex (p < 0.001), there was no statistical difference found in the N1 amplitude over the auditory cortex between groups (p = 0.19).

*Latencies:* The wildtype group exhibited an N1 latency of 36.3 ± 1.6 ms and 49.4 ± 1.6 ms the frontal and auditory channels, respectively. This peak was significantly delayed in the auditory cortex of the mutants with a value of 58.7 ± 0.9 ms (p < 0.01).

**P2 Component**

*Amplitudes:* The P2 amplitude in auditory cortex was 0.14 ± 0.03 μV and 0.04 ± 0.03 μV in the wildtype and mutant groups, respectively. A statistical reduction in the P2 amplitude of auditory AEP was observed in mutants (p < 0.01).

*Latencies:* The wildtype group showed an average P2 latency of 68.9 ± 1.4 ms and 119.4 ± 2.7 ms over the frontal and auditory cortices. The auditory P2 latency in mutants was marginally significantly prolonged with a value of 136.0 ± 1.6 ms (p = 0.051).

**5.2.2 FREQUENCY-DEPENDENT AUDITORY STEADY-STATE RESPONSE**

Figure 16 shows the minimum, maximum, and average ERSP values averaged
across the frontal and auditory channels, respectively, for all the stimulus frequencies. The values were obtained by averaging over the maximal response range for each subject at each group separately. Statistically significant ERSP differences between the PLC-β1 and the control group were observed for both frontal and auditory cortex at all stimulus frequencies (p < 0.05). However, 20 Hz stimulation frequency condition showed the maximum average power for both frontal and auditory cortices for both groups.

To analyze the non-stimulus locked activity deficit in the PLC-β1 group, the baseline power was compared with the power during the stimulus period. The statistics
Figure 16: Comparison of the event related spectral perturbation (ERSP) for the frontal and auditory locations at each stimulus frequency. The markers indicate the average of the maximal response range (0 to 300 ms post-stimulus with a +/- 2 Hz tolerance around the stimulus frequency) for each subject, with the average across subjects indicated by the horizontal bars. The red and black colors are corresponding to the mutant and control groups respectively. The values that are statistically different between the two groups are marked with an * in the x-axis label (p-value<0.05).
of the raw power in the stimulus period (mean and standard deviation) for all stimulus frequencies over left auditory and right auditory channels are shown in figure 17. The white bars show the mean baseline power for the respective conditions. The percent change of the stimulus-locked power from the baseline power is also indicted by the red stem plots on the right y-axis scale. For all stimulus frequencies, the left auditory cortex showed the highest relative power changes for the control group. This channel showed the highest relative power difference between two groups for the 40 Hz stimulus frequency. There was a significant (p < 0.01) increase of baseline power in the PLC-β1(-/-) group compared with the control group over the left auditory cortex at the 40 Hz stimulus frequency.

5.3 DISCUSSION

The PLC-β1 mutants showed significantly attenuated and delayed N1 and P2 responses to auditory tones in the primary auditory cortex and these responses were absent in the frontal cortex. Unlike P1-N1-P2 complex of AEP observed in single or two tone stimuli, the AEP with respect to steady-state stimulus is characterized as N1-P2 complex. N1 in AEP typically reflect stimulus dependent hearing levels and auditory perception and is known to be influenced by frontal cortex activity [93]. Although the best established abnormality in schizophrenia patient is a decrease of the P3a in the frontal cortex in auditory oddball test, the patients with schizophrenia show deficits in early auditory information processing as well, i.e., showing decreased N1 and P2 amplitudes in auditory and frontal cortex [94, 95]. However, this disruption in PLC-β1 mutants is more catastrophic compared to human patients.
Figure 17: Comparison of the average power for the stimulus period and baseline for the left and right auditory channels. The white bars indicate the baseline power overlaid on the corresponding solid bars indicating the stimulus period, with the power scale corresponding to the left y-axis. The error bars indicate the standard deviation of the power during the stimulus period. The red stem plots indicate the resultant percent change in power from the baseline to the stimulus period on the scale provided on the right y-axis.
In rodents, generation of N1 and P2 activities is also inhibited following acute ketamine administration [96] or knockout of Dysbindin-1 or Akt1, another validated candidate genes of schizophrenia [97, 98], however the amount of disruption is significantly smaller compared to AEP responses in PLC-β1 mutants. According to the database of gene expression patterns in the mouse brain presented by Allen Institute (http://www.brain-map.org/), PLC-β1 is highly expressed in all the cortical layers compare to Dysbindin-1 and Akt1. Therefore, the deletion of PLC-β1 in the cortex may lead to more catastrophic disruption of AEP responses. Moreover, all these genes are little expressed in thalamus, except for the thalamic reticular nucleus in case of PLC-β1 [99]. The mid-latency AEP responses are known to reflect sensory gating in paired-tone auditory stimulus studies and thalamic reticular neurons in D-amphetamine treated mice showed no inhibition for the second stimuli in paired-tone auditory stimulus[100]. Therefore the null of PLC-β1 genes in thalamic reticular neurons cannot be opt out as one of the causes of disrupted AEP responses in the systemic null mutant mice. Further validation of the regional knockout of PLC-β1 may lead to identify the main circuit for peak genesis in AEP.
Chapter 6

BRAIN FUNCTIONAL CONNECTIVITY AND SYNCHRONY ANALYSIS DURING AN AUDITORY STEADY STATE STIMULATION IN MOUSE MODELS OF SCHIZOPHRENIA

Schizophrenia patients have shown impairments in the basic sensory processing and higher cognitive functions such as language, reasoning, etc. It is known that these patients have disrupted neural oscillation and connectivity resulting in such sensory and cognitive deficits. Analyzing the brain functional connectivity and synchrony as measures of synaptic plasticity and transmission functions between different brain regions can give insight about healthy brain networks, which can aid the differential diagnosis of neurological impairments such as Schizophrenia, Alzheimers disease, etc. Moreover, measures of synchronization and desynchronization of neural activity can reflect the communication between different areas of the brain, and are representative of synaptic connections and dynamics in the brain circuitry. These oscillatory activities are the fundamental aspect of a normal brain function and are important in the perceptional and cognitional integration of a normal brain [90]. These anatomical oscillatory dysfunctions are dependent on the integrity deficits of the neuronal synaptic activities that are associated with the reduced volume of gray matter [101] as well as white matter [102, 103, 104].
Several studies have been conducted regarding the disruption in the fronto-temporal connectivity and interactions in subjects with schizophrenia [105, 106]. Light et al. (2006) found reduced power and inter-trial phase coherence in response to 30 and 40-Hz frequencies but not to 20-Hz frequency range in schizophrenic patients [107]. Spencer et al. (2008) found a significant phase locking reduction in the schizophrenia patients compared to the controls and this reduction was more obvious in the left hemisphere [108]. Some other studies have also investigated the disconnection syndrome hypothesis of schizophrenia that is the result of improper brain functional integration for such patients [106, 109].

This chapter characterizes the differences between functional brain networks, through different connectivity and synchrony measurement methods, in mice models of PLC-β1(+/+) and PLC-β1(-/-) previously used. To do this, five different synchrony and connectivity measures will be used to assess the likelihood of the reliability of aforementioned methods used for connectivity and synchrony analysis in different brain research studies. Phase locking value (PLV), phase lag index (PLI), directed phase lag index (dPLI) and phase locking factor (PLF) will be used for analyzing the synchrony activity of the brain as well as ADTF method for characterizing the directional information flow rate between different brain regions in such mice models.

6.1 METHODOLOGY

The animal models and data collection procedure are discussed in Chapter 5.
6.1.1 PHASE LOCKING VALUE (PLV)

Phase locking value (PLV) is one of an appropriate and straightforward measure of synchronization for investigating the oscillatory activity of the brain. This method quantifies the interaction between two parts of the brain and demonstrates the cortico-cortical connectivity pattern of the brain at each target frequency \( f \) and each time point \([110, 111]\). In this method, phase synchronization is obtained by the instantaneous phase difference between two time series. Using a combination of Hilbert transform as the imaginary part \( x(t) \) and the real signal \( x(t) \), the analytic form of the signal \( z(t) \) and consequently the instantaneous phase, \( \theta(t) \) can be calculated as below:

\[
z(t) = x(t) + i\tilde{x}(t) = A(t)e^{j\phi(t)}
\]  

\[
A(t) = \sqrt{(|\tilde{x}(t)|^2 + |x(t)|^2)}, \phi(t) = arctan(\tilde{x}(t)/(x(t))
\]  

Therefore, the instantaneous phase difference between two signals is defined as below:

\[
\Delta\theta = \theta_1 - \theta_2
\]

\[
PLV(f,t) \approx \left| \frac{1}{N} \sum_{n=0}^{N-1} e^{i\Delta\theta(f,t,n)} \right|
\]
where $N$ is the total number of trials and $\Delta \theta (f, t, n)$ is the phase difference between two signals at the target frequency $f$ and time point $t$ and the $n$th trial. PLV can vary between 1 to 0 which shows whether the two signals are perfectly phase locked or not.

### 6.1.2 PHASE LAG INDEX (PLI)

It has been shown that different types of reference electrodes and volume conduction can cause an error in the true estimation of phase synchronization between two brain signals recorded at two different places [112, 113]. However, in this measure of synchrony (PLV), any linear mixing of the activity coming from the common sources can cause the fake non-zero phase locking between two signals which is undesirable. To overcome this problem, Stam et al. (2007) proposed a new measure of phase synchrony called phase lag index (PLI) that is invariant under the presence of common sources and is defined as below [114]:

$$PLI (f, t) \approx \left| \frac{1}{N} \sum_{n=0}^{N-1} \text{sign} (\Delta \theta (f, t, n)) \right|$$

(17)

This synchrony measurement is quantified by the asymmetry of the distribution of phase differences centered at zero. If there is no phase coupling between two signals, this distribution is flat and any deviation from flatness shows the phase synchronization between the two signals. If the number of trials with $-\pi < \Delta \theta < 0$ is equal to the number of trials with $0 < \Delta \theta < \pi$, then the distribution would be symmetric which shows zero phase lag between two signals. Any skewness toward
left or right shows a non-zero phase lag and the more the skewness is (either negative or positive), the more the two signals are coupled with each other.

6.1.3 DIRECTED PHASE LAG INDEX (DPLI)

Although PLI can overcome the spurious synchronized problem caused by volume conduction and common source activity, it does not give us any information about the direction of the phase difference distribution skewness that can show which channel was leading and which channel was lagging with respect to the other channels. Directed phase lag index (dPLI) is the modified version of PLI which can give us insight about the sign of phase difference between two time series. Let us suppose that the phase difference is \( \Delta \theta = \theta_1 - \theta_2 \) [115]. Then, the dPLI between two time series \( x \) and \( y \) can be defined as below:

\[
dPLI_{xy}(f, t) = \frac{1}{N} \sum_{t=1}^{N} H(\Delta \theta(n, t, f))
\]  

(18)

Where \( H \) is Heaviside step function. Any skewness toward right or left shows dPLI \( \neq 0.5 \). In case that in half of the trials one channel was leading and in another half of the trials the same channel was lagging with respect to the other channel, dPLI would be equal to 0.5 which shows that neither of the respective two channels was leading or lagging. If dPLI > 0.5, it means that time series \( x \) is leading and if dPLI < 0.5, it means that \( x \) was lagging. Moreover, if dPLI = 0.5, PLI is 0; and if dPLI is 1 or 0, PLI is 1. We can show the relation between these two types of synchrony measurement as below:
\[ PLI = 2|0.5 - dPLI| \]  

This shows that if we have dPLI, we can obtain PLI but vice versa is not achievable.

### 6.1.4 PHASE-LOCKING FACTOR (PLF)

The phase-locking factor (PLF) is a special case of PLV for evaluating synchronization of a signal with a reference stimulus. In this case, one of the signals beginning compared simply represents the stimulus signal. The signals from each channel were filtered using a zero-phase FIR filters with 2-Hz bandwidth centered at frequencies from 15-55 Hz in 1-Hz increments. The Hilbert transform was used to compute the instantaneous phases of the resulting narrow-band signals and then PLV, PLI, dPLI and PLF were computed at all stimulus frequencies and channel pair combinations during the stimulus period.

### 6.1.5 ADAPTIVE DIRECTED TRANSFER FUNCTION (ADTF)

The adaptive directed transfer function (ADTF) provides a measure of the information flow rate between different parts of the brain and this type of measurement tends to be more complicated than other synchrony measurements. The results were compared between the PLC-\(\beta1(+/+)\) and PLC-\(\beta1(-/-)\) groups. Directional connectivity analysis was performed on the auditory evoked potentials (AEPs), after they were bandpass filtered in the frequency range of 5-55 Hz. The maximum of the
autoregressive model was set to 12. The statistical test was performed using 200 surrogate samples with a significance level of $\alpha = 0.05$. The significant and the non-significant ADTF values were set to 1 and 0, respectively, to show the percentage of subjects with significant connectivity values for each connection, frequency, and time point.

6.1.6 STATISTICAL ANALYSIS

Kruskal-Wallis non-parametric one-way analysis of variance test was performed separately for each measurement (PLV, PLI, dPLI, PLF) to evaluate the statistically significant differences between the two groups. The statistical analysis was performed for each channel or connection at each stimulus frequency separately with a significance level of alpha=0.05.

6.2 RESULTS

6.2.1 PLV

The average PLV values in the maximal response range (0 to 300 ms post-stimulus with a +/- 2 Hz tolerance around the stimulus frequency) are shown in 18 for the PLC-\(\beta\)1 and control groups at the 20, 30, 40 and 50 Hz gamma stimulus frequencies. The thickness of the line indicates the magnitude of cortico-cortical locking value, with thicker lines indicating higher phase locking synchronization between connections. The frontal connection (LF-RF) exhibited the highest synchrony to the task in
both groups at all the stimulus frequencies. This connection had its highest synchronized activity during stimulus frequency of 20 Hz with the mean PLV magnitudes of $0.7241 \pm 0.07$ and $0.6131 \pm 0.049$ for the healthy and PLC-β1 groups, respectively. This value for 40 Hz gamma frequency band were $0.62 \pm 0.08$ and $0.53 \pm 0.04$ for both groups of control and PLC-β1 groups. Using a Kruskal-Wallis test, this connection showed a statistically significant difference ($p < 0.0001$) between the two groups for all the stimulus frequencies. Although the connection between the two auditory sites (LA-RA) showed a significantly decreased phase synchronization in PLC-β1 group compared to the control group ($p < 0.01$) for the 40Hz gamma stimulus frequency, this connection showed the least overall synchrony across groups with PLV magnitudes of $0.26 \pm 0.16$ and $0.13 \pm 0.04$ for healthy control and PLC-β1 groups, respectively. While the cortico-cortical locking analysis showed that the synchrony for all connections to the left auditory cortex (LA) was significantly reduced in PLC-β1 group for all the stimulus frequencies, the synchronized activity between right auditory cortex (RA) and the frontal channels did not show significant disruption for this group at gamma stimulus frequencies of 30, 40, and 50 Hz. This can be indicative of more synchronized neural impairment on the left auditory cortex than the right auditory cortex in the PLC-β1 groups at gamma stimulus frequency. The significant impairment of cortico-cortical phase locking activity between LA and all other connections for PLC-β1 group was consistent among all the stimulus frequencies ($p < 0.05$).
Figure 18: The average phase locking value (PLV) connectivity for all the stimulus frequencies. The line widths correspond to the PLV connectivity, with thicker corresponding to larger PLV. The circles indicate the connections that are significantly reduced in the PLC $\beta 1$ (-/-) group compared to the control group (p <0.05).
6.2.2 PLI

Similar to the PLV analysis, the average PLI values in the maximal response range were calculated at all the stimulus frequencies for both groups. Despite the higher synchronization between the two frontal channels using PLV, PLI showed a comparatively reduced synchronization between the two frontal channels. The spurious high synchrony activity of LF and RF obtained by PLV can possibly be explained as the result of volume conduction. Since these two frontal channels were recorded from the surface of the skull while the two auditory electrodes were implanted in the cortex, the PLV values for LA and RA channels are less susceptible to volume conduction effects that can result in spurious connectivity estimates. As it is shown in figure 19, the connection from left auditory (LA) to left frontal (LF) cortex has the highest synchronized activity during all the stimulus frequencies, with the highest synchrony response at gamma stimulus frequency of 40 Hz with the mean values of 0.125 ± 0.05 and 0.06 ± 0.03 for the control and PLC-β1 groups, respectively.

6.2.3 DPLI

Directional neural coordination was calculated using the dPLI measurement for both groups at all the stimulus frequencies, and the average values over the maximal response range is shown in figure 20. As is shown, the phase difference between the LA and the two frontal channels was higher for the control group at all the stimulus frequencies compared with the other connections with the highest value being 0.06 ± 0.02 for the LF-LA connection for the control group. This shows that for the
Figure 19: The average phase lag index (PLI) connectivity for all the stimulus frequencies. The line widths correspond to the PLI connectivity, with thicker corresponding to larger PLI. The circles indicate the connections that are significantly reduced in the PLC-β1 (-/-) group compared to the control group (p <0.05).
Figure 20: The average directional phase lag index (dPLI) for all the stimulus frequencies.
40 Hz ASSR

**PLC β1 (+/+)**

![Graph of PLC β1 (+/+) showing phase differences and frequency dimensions](image)

**PLC β1 (−/−)**

![Graph of PLC β1 (−/−) showing phase differences and frequency dimensions](image)

Figure 21: Time-frequency map of dPLI for the 40 Hz stimulus frequency for both groups.
control group, the frontal channels are substantially leading in phase with respect to the LA channel, compared to the other channels. However, not only was this pattern not observed in the PLC-$\beta_1(-/-)$ group, but this group also did not show any consistent phase lead or lag for any channel pair or stimulus frequency. This can possibly be explained by the associations of disrupted neural coordination with auditory hallucination and disorganized brain network in patients with Schizophrenia impairment [116]. The directional phase synchrony from each pair of channels from auditory to frontal for both groups did not show any comparative lag in phase. This shows that there was no phase shift in the ASSR between the auditory channels or the frontal channels, at any stimulus frequency, for either groups.

By applying a Kruskal-Wallis test, a significant reduction in the phase shift of the LA and two frontal channels for the PLC-$\beta_1(-/-)$ group compared to the healthy subjects was seen ($p < 0.00001$) at all the stimulus frequencies. However, no significant reduction in phase lead was seen in the directional synchrony response from frontal channels to the RA cortex in the PLC-$\beta_1/-$ groups compared to the control subjects. Figure 21 shows the time-frequency map of dPLI for all the pre- and post-stimulus period over all the frequencies of 15-55 Hz for the 40 Hz stimulus frequency.

6.2.4 PLF

The phase-locked auditory steady-state response during the stimulation period was reduced in the PLCbeta1 null mice for both auditory and frontal cortex at all the stimulation frequencies. However, for the 20Hz and 40Hz stimulus frequencies, the left auditory cortex revealed a significant reduction in the phase locking to the
Figure 22: Comparison of phase locking factor (PLF) for the frontal and auditory locations at each stimulus frequency. The markers indicate the average of the maximal response range (0 to 300 ms post-stimulus with a +/- 2 Hz tolerance around the stimulus frequency) for each subject, with the average across subjects indicated by the horizontal bars. The red and black colors are corresponding to the mutant and control groups respectively. The values that are statistically different between the two groups are marked with an * in the x-axis label (p-value<0.05).
stimulation for the PLC-β1(-/-) groups (p<0.05, p<0.01 respectively) compared to the control group. The other stimulus frequencies did not show a significant difference between the two groups at any channel. However, the two frontal channels also had significant decrease at the 20 Hz stimulus frequency for the PLC-β1 null group compared with the control group. Figure 22 shows the minimum, maximum, and average PLF values averaged across the frontal and auditory channels, respectively, for all the stimulus frequencies. The values were obtained by averaging over the maximal response range for each subject at each group separately. The highest synchronized activity for both groups was observed in the frontal cortex for the 20 Hz stimulus frequency with the mean values of 0.19 ± 0.09 and 0.11 ± 0.04 for control and PLC-β1 group, respectively. This activity was significantly disrupted in the PLCbeta1 null group (p < 0.05). In general, the average phase locking factors

Figure 23: The average phase locking factor (PLF) time-frequency maps for the 40 Hz stimulus frequency. The vertical white bars indicate the beginning and end of the stimulus period. The plots are arranged topographically for each group according to frontal/auditory and left/right.
over the maximal response range for both groups were higher in the frontal compared with the auditory cortex in the gamma band of 40 Hz (PLC-β1(+/+): 0.11 ± 0.05, 0.08 ± 0.03 and PLC-β(-/-): 0.07 ± 0.02, 0.04 ± 0.01 for the frontal and auditory cortices respectively). This shows that the frontal cortex was more synchronized with the auditory stimulus click train than the auditory cortex for both groups, which was also consistent across all stimulus frequencies. Figure 23 shows the average PLF time-frequency maps for the 40 Hz stimulus. While it is clear that there is reduced activity in all channels at 40 Hz during the stimulus period for the PLC-β1 null mice compared to the control group, phase locking to the stimulus was not significantly altered in the right auditory cortex or the frontal cortex. The 40 Hz PLF in the right auditory cortex of control mice was also found to be reduced compared to the left auditory cortex.

6.2.5 ADFT

Figure 24 and 25 show the percentages of subjects with significant connectivity between two different parts of the brain, separately for each group and stimulus frequency. As can be seen, for most of the stimulus frequencies, there is a high bidirectional information flow rate between the two channels located in the auditory and the frontal cortex, for both groups. However, at the 40 Hz gamma stimulus frequency, there is more spread in the pattern of connectivity, with the highest percentage of significant information flow rate observed over the stimulus period for the PLC-β1(-/-) group, compared to the control group, in two auditory and frontal bidirectional connections. The pattern of connectivity for all the stimulus frequencies was almost
consistent for the control group. In contrast, this pattern shows more spread in the connectivity pattern at the 40 Hz gamma stimulus frequency, which has shown the most disrupted synchrony activity in an ASSR task. For all the stimulus frequencies, it can be seen that the percentage of control subjects with significant information flow rate, is higher from the auditory cortex to the frontal channels, in comparison with the opposite direction (frontal to auditory). However, the bidirectional connectivity pattern between the frontal and the auditory cortex for the PLC-β1(-/-) group, does not show any noticeable similarity to the control subjects.

6.3 DISCUSSION

Overall, the present results were consistent with the ASSR of human patients with schizophrenia showing reduction in the phase locking of 40 Hz in the left auditory cortex and cortico-cortical connectivity at 40 Hz. However, we also found several findings inconsistent with the human data. First, in human patients, the power and phase locking of ASSR were impaired selectively at 40 Hz stimulation [88, 108]. In our study, however, the power of ASSR was reduced in all the test frequencies. We observed frequency selective reduction in the phase locking in mutants, however, differently from previous reports, the deficit was more significant at 20 Hz than at 40 Hz. More interestingly, in frontal cortex, the phase locking was not impaired at 40 Hz, but decreased at 20 Hz. Although in some studies, the deficits of neighboring frequencies (e.g., 30 Hz) were also observed [117, 107], 20 Hz ASSRs are usually unaffected in patients with schizophrenia. Similarly to our results, Nakao, et al., observed 20 Hz deficits in ASSR power and phase locking factor in the auditory
cortex of the interneuron-specific NMDA receptor hypofunction mice, however, the degree of ASSR deficits at 40 Hz was more robust than 20 Hz. The most prevailing explanation of pathologic origin of schizophrenia is NMDA receptor hypofunction, inducing downregulation of parvalbumin-positive cortical GABAergic neurons, pyramidal neuron dendritic dysgenesis, and reduced spine density [118]. Although there is no direct link between PLC-β1 signaling and NMDA receptors, a morphological study has shown that the spine density in the cortical neurons of PLC-β1 mutants is reduced because a lacking of PLC-β1 signaling inhibits the maturation of dendritic spines [119]. This reduced synapse per neuron ratio indicates an overall decrease in the synaptic connectivity, and it is conceivable that the frequency-independent reduction in ASSR powers could be due to low synapse levels in the mutants. On the other hand, although the left auditory cortex showed disruption of PLF in 40 Hz, no disruption of PLF at 40 Hz was observed in neither left nor right frontal cortex. Animal studies have shown that the frequency specificity of 40 Hz is related to parvalbumin (PV)-positive fast-spiking interneurons, whose intrinsic resonance near this range [120] affirmed by optogenetic method [121, 39]. Moreover, recently, Tae et al. showed the cortical projecting basal forebrain PV neurons generates cortical oscillation preferentially to 40 Hz via synchronizing the cortical PV interneurons [122], which is consistent with abnormality of cortical interneurons in schizophrenia patients [34]. Therefore, our observation of the intact 40 Hz ASSR in frontal cortex may rule out the relevance between PLC-β1 disruption and abnormalities in
cortical PV interneurons. Instead, PLC-\(\beta1\) has been known to be linked to muscarinic acetylcholine receptors [123], which regulates GABA receptor inhibition in prefrontal cortex [32], suggesting indirect regulation of PLC-\(\beta1\) in the GABAergic receptors functioning in prefrontal cortex [30]. Therefore, the absence 20 Hz specific disruption of PLF is speculated to be related to the muscarinic acetylcholine receptor affiliated GABAergic circuits not related to fast-spiking interneurons, limiting the pathophysiological relevance of PLC-\(\beta1\) mutants as schizophrenia model. Furthermore, in human patients with chronic schizophrenia, the level of PLC-\(\beta1\) proteins increased in prefrontal cortex and decreased in the superior temporal gyrus [124].

Interestingly, the reduction in power and disruption in phase synchronization are stronger in the left hemisphere in mutants, whereas the AEP showed the similar levels in left and right hemisphere (data not shown). In human patients, the disrupted ASSR synchrony in the left auditory cortex was positively correlated to the severity of auditory hallucination symptoms [125]. Handedness does not appear to be related to this hemispheric relationship to hallucination because unlike human, the mice are prone to be sinistral or mixed [126]. Along with this left-hemisphere predominant neurophysiology, neuroanatomical asymmetries are known to be present in the brain of patients with schizophrenia. Onitsuka, et al. showed that the gray matter volume in the middle temporal gyrus of patients with schizophrenia is reduced more in the left hemisphere compared to the right hemisphere [127]. Furthermore, O’donnell et al. showed that the decreased gray matter in the left auditory cortex is linked to the auditory P300 visual evoked potential deficit in the schizophrenia patients [128].
In addition, a deterioration of PLC-β1 cascade has been found in the left, but not right superior temporal gyrus of patients with schizophrenia showing neurochemical evidence for the asymmetry of the temporal lobe of schizophrenia [129]. The reduction of gray matter implicates a reduction in the synchronization activities of the cortical neurons, and our observation of left-hemisphere predominant disruption of ASSR in terms of power, phase locking, and cortico-cortical connectivity are in line with these neuroanatomical and neurochemical observations. However, in mice, no evidence has been reported in regards to the asymmetric expression of PLC-β1 genes to date. Considering PLC-β1 signaling pathway play a role in the development of normal cortical circuitry [119], it is speculated that PLC-β1 knockout mice has disrupted functional connectivity as well.
Figure 24: The percentage of significant ADTF values for the control and PLC-β1(+/−) groups at stimulus frequencies of 20 and 30 Hz. The direction of information flow is indicated above each subplot.
Figure 25: The percentage of significant ADTF values for the control and PLC-β1(-/-) groups at stimulus frequencies of 40 and 50 Hz. The direction of information flow is indicated above each subplot.
Chapter 7

CONCLUSION AND FUTURE WORK

This dissertation consists of multiple scientific investigations related to characterizing EEG features and neural connectivity for two prevalent neurological impairments, namely ALS and schizophrenia. The findings are envisioned to lead to better diagnostic techniques and interventions for these patient groups. Furthermore, the methodologies and findings can be extended to characterizing neurological impairments for similar and related neurological conditions. This chapter summarizes the most significant findings and key contributions of this dissertation. It concludes with a discussion of possible future directions and extensions of this work.

7.1 KEY CONTRIBUTIONS

7.1.1 CHARACTERIZATION AND APPLICATION OF EEG FEATURES AND CONNECTIVITY IN ALS PATIENTS

The significant correlations of temporal and spectral EEG features with BCI performance, for individuals with ALS, were explored using a unique longitudinal dataset. This work provides a novel characterization of the P300-BCI performance variations between ALS subjects. The results show that the delta-band activity recorded primarily from occipital locations was significantly negatively correlated with BCI performance in 89% of the users and the alpha-band activity recorded
primarily at central and occipital locations was significantly positively correlated with performance in the same percentage of users. Additionally, 78% of the users exhibited a positive deflection in EEG amplitude around 220 ms at frontal midline locations (i.e., Fz and Cz) that had significant positive correlation with performance. These findings can inspire methods to improve BCI reliability and lead to a more robust communication system with the goal of providing assistance such patients.

Since the primary reason for exploring the correlation of EEG features with performance variations is to develop a predictive model for evaluating the BCI readiness of ALS subjects before conducting the complete BCI session, several performance prediction algorithms were tested using these features. Using both spectral and temporal features, MLP and linear SVM classifiers showed the best prediction performance results (i.e. accuracy = 66%), which were well above chance accuracy (chance accuracy = 50%). Although these preliminary results are promising, the prediction performance can potentially be enhanced using more sophisticated feature selection and machine learning algorithms.

To further characterize BCI performance variations in ALS patients, the causality between EEG electrode locations during use of a P300-based BCI was investigated. The results show that there are statistically significant causal relationships between channels, particularly in the high beta frequency range, that are consistent across subject groups. The connectivity patterns in the ALS group appeared to be more diffused when compared to the control group. This can possibly be explained as the loss of inhibitory neurons in such patients that causes greater spread in the
pattern of connectivity. The highest percentage of connectivity was observed from the feedback connection of Fz to Cz for both groups. These findings suggest that there are differences in brain activity between individuals with and without ALS, as well as in the activity across successful and unsuccessful task sessions, in a particular individual, while using a P300-based BCI. This knowledge can lead to improved methods to assess and improve BCI performance for all BCI users.

7.1.2 CHARACTERIZATION OF EEG FEATURES AND CONNECTIVITY IN MOUSE MODELS OF SCHIZOPHRENIA

The second half of this dissertation explores the impairments of ASSS responses in a mutant mouse model of schizophrenia in comparison to healthy mice controls. A late and reduced AEP was observed in the schizophrenic mice models compared with the healthy group. In fact, these mutant models showed significantly disrupted and delayed N1 and P2 components of AEP in response to ASSS in the auditory cortex. These delayed responses were not seen in the frontal cortex.

In addition to morphological investigations, the power spectra were also explored using ERSP at each channel location. Significantly higher power spectral values were observed in all the stimulus frequencies, for the control group, as compared to the mutant mice. Non-stimulus locked activity was also analyzed with respect to the baseline response and significantly higher baseline activity was observed at the 40 Hz gamma frequency band in the mutant models. These results can be indicative of a disruption in the brain sensory processing in such patients, which can lead to auditory hallucinations and impaired auditory perception. One of the unique aspects
of this dataset is the use of animal models, which provides histological information that can link the electrophysiological markers to structural differences.

The characterization of functional brain network connectivity and synchrony in the mutant mice is another key contribution of this work. Based on several connectivity and synchrony measurement methods, significantly disrupted brain functional connectivity and synchrony pattern was observed in the mutants. For the ADTF connectivity analysis, the results showed more spread in the connectivity pattern in the mutant types, as compared to the controls, at a gamma frequency of 40 Hz. This can possibly be attributed to the dysfunction of GABAergic neurotransmitters, or the over activity of dopamine D1 receptors in such patients.

The coordinated neural activity of these mutant animal models were investigated using different synchrony measurement methods, in order to evaluate their advantages. The analysis using PLV, showed that left auditory (LA) cortex had the most synchrony disruption in mutants. However, a strong coordinated neural activity was observed in both groups, between the two frontal channels, also using PLV. In contrast, the introduction of the PLI measurement method did not exhibit this frontal connectivity, which is presumed to be caused by volume conduction or referencing issues.

When analyzing the phase synchrony between pairs of channels, PLV and PLI do not provide information regarding phase lead or lag. To overcome this problem, dPLI was introduced, which measures the directional synchronization between two locations. The results showed that the frontal channels were significantly leading in
phase the left auditory channel, in the control group but not in the mutants. These findings can be indicative of impaired neural coordination in the left auditory cortex as a measure of auditory hallucinations, in the schizophrenia mice models.

The contribution of this work is the introduction of novel electrophysiological markers for describing neural deficits in the schizophrenia model. This can contribute to a better understanding of the associations between higher functional impairments of perception, working memory, cognition etc., and brain synchrony in schizophrenia. This can, in turn lead to the development of a clinically relevant model of cortical changes in such patients, and ideally, towards the development of biofeedback techniques for neurotherapy.

7.2 FUTURE DIRECTIONS

7.2.1 CHARACTERIZATION AND APPLICATION OF EEG FEATURES AND CONNECTIVITY IN ALS PATIENTS

Although simple spectral and temporal features for predicting BCI performance variations in ALS patients provided promising results that were well above chance accuracy, these accuracies are still not sufficient for practical application. More sophisticated feature extraction, feature selection, and classification algorithms should be investigated to further improve the P300 performance variation predictions.

Aside from the connectivity analysis approaches used in this work, other connectivity and synchrony measurement methods such as Cross-frequency coupling (CFC).
Coherence analysis, Granger causality, PLV, etc., should be implemented and compared, to see if they can supplement the understanding of discriminatory power and therefore, lead to a preferred approach.

One hypothesis to explain P300 performance variations in ALS patients, is that the signal is more non-stationary during unsuccessful sessions, which results in a less accurate classification performance. Therefore, investigating this issue using different methods such as stationary subspace analysis (SSA) and analytic SSA (ASSA) is encouraged. However, one challenge with SSA is optimizing the parameters such as the number of stationary sources, epoch lengths, etc., which can provide misleading outcomes, if they are not properly selected.

The outcomes related to predicting the P300 performance variations in ALS subjects, can be expanded and applied to different BCI applications, such as those that use sensorimotor and steady-state signals.

Since an important practical outcome is to predict the P300-BCI readiness of BCI users with ALS, the development of a real-time machine learning algorithm for this problem will be the most challenging and impactful extension of this work.

### 7.2.2 CHARACTERIZATION OF EEG FEATURES AND CONNECTIVITY IN MOUSE MODELS OF SCHIZOPHRENIA

For the synchrony and connectivity analysis of the ALS data, other approaches such as Granger causality, Partial directed coherence (PDC), Coherence, etc., can be of interest to investigate the generalizability of the results and development of a more preferred approach.
Anatomical connectivity analysis in addition to functional analysis can contribute to a better understanding of structural and functional relationships between different parts of the brain. This can be done through graph analysis. In fact, investigating the structural network analysis of neurological impairments can provide information about local and global brain processing disruption and, therefore, emergent functional abnormalities in schizophrenia patients. This can contribute to the development of a more accurate optimal brain topology and, thus, a more powerful biomarker for diagnosis of the respective disease.

Based on the resulting characterization outcomes for mice models of schizophrenia, in conjunction with the aforementioned future work, the evaluation of different target drugs on such mutant populations is recommended. The efficiency of each medication can be evaluated based on achieving similar brain functional patterns between the mutant and healthy control. This could eventually lead to better treatment of schizophrenia in humans.
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Selected Publications
4. Y. Shahriari, A. Erfanian, Mutual Information Based Brain Channel Selection Scheme For P300- Based Brain Computer Interface, Presented in the 5th International IEEE EMBS on Neural Engineering, 2011.

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