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Seed-based Functional Connectivity Analysis of Hippocampal Network of Patients Suffering from Major Depressive Disorder

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Preface

The basis for this project stemmed from the fact that not many researches have been conducted in Nepal regarding the diagnosis of mental disorders. Nonetheless, in other countries, many researches and studies have been conducted regarding the functional connectivity of different brain regions in depression and other mental disorders. However, till date, there is no solid evidence that could be used for the clinical diagnosis of mental disorders. Our project intends to review past researches and keep up with the studies related to Major Depressive Disorder and brain functional connectivity. In addition to that, we have selected hippocampal circuity as the region of interest for our purposes and the overall project is going to revolve around how functional connectivity of hippocampal network in MDD patients differ from that of healthy people.

The following proposal begins with the basic concepts of the resting state functional connectivity of brain, different regions of brain and MR images. The concept of MR image processing, functional connectivity analysis will be used in the project. Making use of these concepts, our project approaches to compare and come up with a conclusion about how functional connectivity differs in MDD patients and how the findings of the project could play a role in diagnosis of MDD. In this project, the use of "functional connectivity" is restricted to mean quantification of the operational interactions of multiple spatially distinct brain regions that are not engaged in any specific task or stimulus. We will further restrict our discussion to connectivity measures of the hippocampal network, derived from fMRI imaging modality alone. We are required to conduct this project as a part of the curriculum of Biomedical engineering. We hope to acquire a profound knowledge about MR image processing which would help us a lot in our career as biomedical engineers.

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Abstract

The absence of biological markers makes it exceptionally difficult for neurologists to diagnose a person with a mental disorder. Currently, diagnosis of mental disorders is based on behavioral observations and patient-reported symptoms, both of which do not have a molecular or an imaging basis. Although there have been thousands of studies revolving around the implementation of various imaging modalities for deciphering the etiology and the physical cause of several mental disorders, the findings from these studies do not appear amongst the diagnostic criteria. Meaning that the findings from these studies are not used for diagnosis purposes. A critical barrier to the clinical translation of many such findings is the reverse inference fallacy, as neurological disorders are multifaceted and are influenced by more than one factor and neuroimaging results can be heavily influenced by external factors such as patient movement and instrumental artifacts. However, neuroimaging for diagnosis of mental disorders seems promising in the future and as a matter of fact, a small minority of health care providers have already started to implement neuroimaging techniques such as fMRI, SPECT, PET for the diagnosis of psychiatric disorders. Nevertheless, there is no solid molecular or imaging basis that is widely accepted for the assessment of mental disorders. Here in the proposed research we plan to evaluate functional network connectivity of the hippocampus in patients with Major Depressive Disorder based on the MR images of their brain and investigate the changes in the brain network compared to that of healthy controls matched according to age and gender.

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Abbreviations

ACC Anterior Cingulate Cortex

AFNI Analysis of Functional Neuroimages

ASN Anterior Salience network

BDI Beck Depression Inventory

BOLD Blood Oxygen Level Dependent

CA Cornu Ammonis

DG Dentate Gyrus

DMN Default Mode Network

DSM Diagnostic and Statistical Manual of Mental Disorders

EC Entorhinal Cortex

ECN Executive Control Network

EEG Electroencephalography

fMRI Functional Magnetic Resonance Imaging HC Healthy Controls

HPA Hypothalamic Pituitary Adrenal

ICA Independent Components Analysis

ICD International Classification for Diseases

MDD Major Depressive Disorder

MFC Medial Frontal Cortex

MFG Middle Frontal Gyrus

MR Magnetic Resonance

OFC Orbitofrontal Cortex

PET Positron Emission Tomography

PMC Premotor Cortex

rFPDD Recurrent Familial Pure Depressive Disorder

ROI Region of Interest

rs Resting State

rsFC Resting-state Functional Connectivity

SCA Seed-based Correlation Analysis

SPECT Single-Photon Emission Computed Tomography

sMRI Structural Magnetic Resonance Imaging

SNRI Serotonin and Norepinephrine Reuptake Inhibitors

SSRIs Selective Serotonin Reuptake Inhibitors

vPFC Ventrolateral Prefrontal Cortex

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1 Introduction

1.1 Major Depressive Disorder

Major Depressive Disorder, generally abbreviated as MDD, is one of the most common and serious mental disorders. MDD is also referred to as clinical depression, or just depression as well. MDD can be characterized by an array of distinct symptoms, some of them being persistent feelings of sadness, feelings of low self-worth and guilt, and overall reduced ability to take pleasure from activities that previously were enjoyable and so on. Although, the exact symptoms of depression may vary from person to person depending on their upbringing and various socio-demographic variables such as age, sex, religious affiliations, employment, income etc. For an individual to be classified as "suffering from MDD", five out of ten symptoms, one from a set of two and four additional symptoms from another set of five, must be present during a span of two weeks **whodepression**. In addition to the symptoms that may be prevalent in a person suffering from depression, there can be morphological differences in several brain regions including the frontal and temporal lobes. On top of that, individuals suffering from MDD also have abnormal functional connectivity. According to the World Health Organization, more than 264 million people of all ages suffer from depression worldwide **whodepression**. Fortunately, there are effective psychological and pharmacological treatments for moderate to severe depression. The pharmacological treatment includes medications such as the SSRIs and SNRI which are two most commonly prescribed antidepressants. The psychological treatments include psychotherapy and electroconvulsive therapy, depending on the severity of the depression.

1.2 Neuroimaging

Neuroimaging or brain imaging is the use of various imaging modalities to either directly or indirectly image the structure, function, or pharmacology of the nervous system. Current neuroimaging techniques reveal both form and function. They reveal the brain's anatomy, including the integrity of brain structures and their interconnections. Diagnostic neuroimaging has two prospects, one is short-term prospects and the other is long-term prospects. The near-term or short-term prospects of diagnostic neuroimaging allows validation of the categories of neuro-disorders rather than in the criteria for diagnosing an individual patient. On the other hand, long term prospects allow diagnostic distinctions that are difficult to make on the basis of behavioral observations alone, so potentially distinctive patterns of brain activation identified through imaging will be useful. Neuroimaging can be divided into two broad categories:

1. **Structural Imaging**, which deals with the structure of the nervous system and the diagnosis of gross (large scale) intracranial disease (such as a tumor) and head injury.

2. **Functional imaging**, which is used to diagnose metabolic diseases and lesions on a finer scale (such as Alzheimer's disease) and also for neurological and cognitive psychology research and building brain-computer interfaces **neuroimaging**.

Functional Magnetic resonance imaging (fMRI) is a modern technique of imaging, which is a powerful non-invasive and safe tool used for the study of the function of the brain based on the measure of the brain neural activation. The fMRI can localize the location of activity in the brain which is caused due to sensory stimulation or cognitive function. In the clinical setting, fMRI allows the researchers to study how healthy brain functions, how different diseases affect the brain functions, how brain functions altered due to disease or injury can be restored, and how drugs can control the disease's effect on brain activity **fMRI**.

1.3 Brain Networks

A brain network, on a large scale, can be defined as a collection of brain regions working together to produce a specific function. Brain networks can be identified at different resolutions, therefore there is no universal atlas of brain networks that fits all circumstances. However, based on converging evidence from related studies, there are six large-scale, core brain networks that are most widely accepted due to their stability **estimatingLargeScaleNetworks**. The following figure is a diagrammatic representation of the core brain networks:

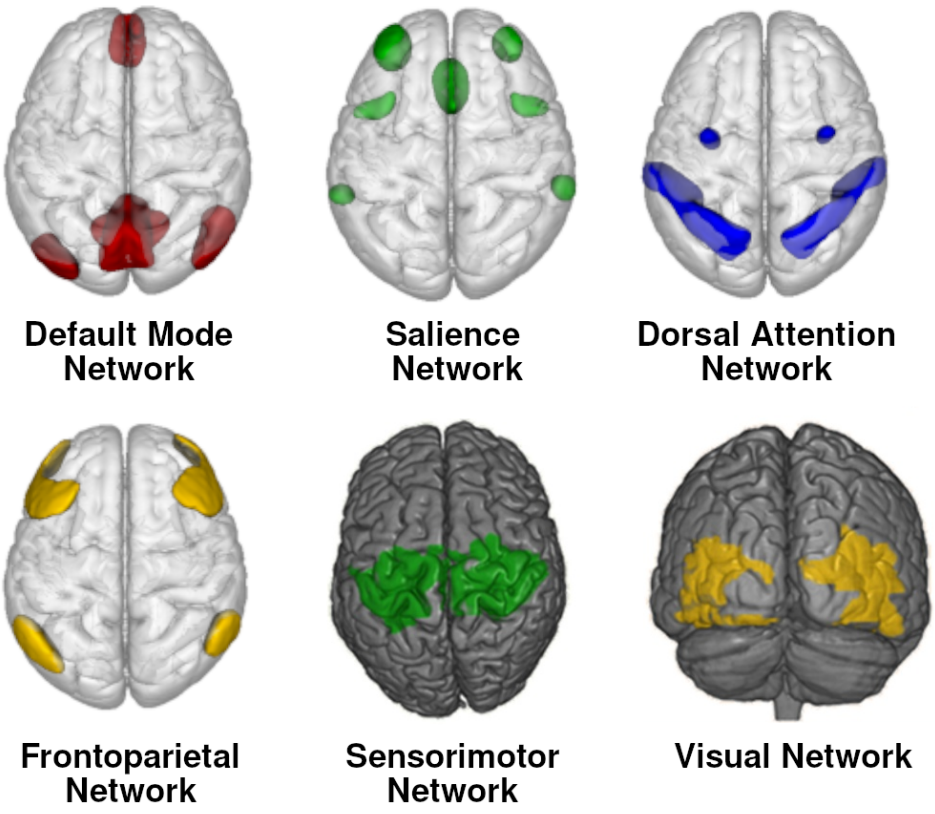

Figure 1: Core Brain Networks
dorsalattentionfrontoMajorbrainnetworks

1. **Default Mode Network:** This network is active when an individual is awake and at rest **serendipitousDMN**.

2. **Salience Network:** Monitors the salience of external inputs and internal brain networks **saliencemetabolicfunctionalconnectivity**.

3. **Dorsal Attention Network:** Involved in voluntary, top-down deployment of attention **thomasorganization**.

4. **Frontoparietal Attention Network:** Initiates and modulates cognitive control **frontoparietalAttention**

5. **Sensorimotor Network:** Processes somatosensory information and coordinates motion **heine2012resting**.

6. **Visual Network:** This network handles visual information processing **brainvisual**.

There are more subsets of these six networks such as the limbic, auditory, right/left executive, cerebellar, spatial attention, language, lateral visual, temporal and visual perception/imagery. An emerging paradigm in neuroscience is that cognitive tasks are performed not by individual brain regions working in isolation but rather by brain networks consisting of several discrete brain regions that are said to be "functionally connected". The functional connectivity of brain networks can be acknowledged through (statistical) analysis of images acquired through a variety of techniques such as the fMRI, EEG, PET or SPECT **wikibrainnetworks**.

1.4 Resting State Functional Connectivity

Functional connectivity can be defined as the temporal correlation between spatially remote neurophysiological events, expressed as deviation from statistical independence across these events in distributed neuronal groups and areas. In simple terms, functional connectivity refers to the communication be tween functionally distinct brain regions **functionalConnectivityNikin**. The results of the study conducted by Bharat B. Biswal et. al. suggests that while variations in blood flow might contribute to functional connectivity maps, BOLD signals play a dominant role in the mechanism that gives rise to functional connectivity in the human brain **biswalsimultaneous**. During resting conditions, our brain remains functionally and metabolically active. The fact that brain remains "metabolically active" means there will be consumption of oxygen which results in fluctuations in the BOLD signal. Spontaneous fluctuations in distinct brain regions show temporal correlations with each other, reveling complex patterns of functional connectivity **biswal1995functional**.

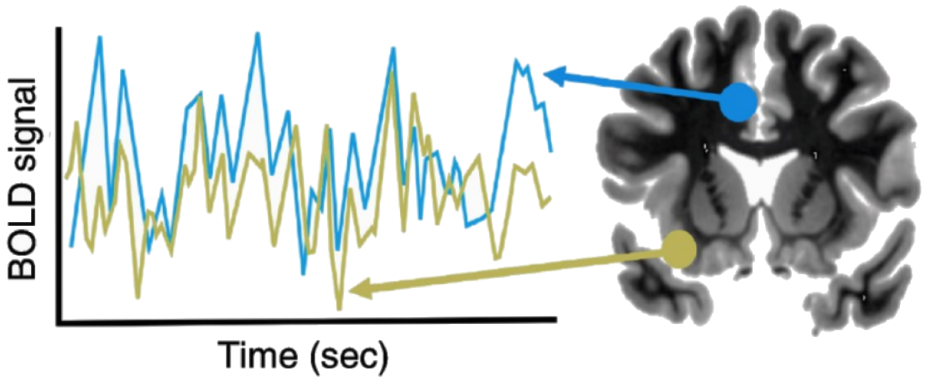

Figure 2: Schematic Representation of Bold Signal Fluctuations

In the above diagram, the solid blue line represents the BOLD signal of one brain network and the green line represents that of another brain network. Now, the resting state functional connectivity can be defined as the correlation patterns in the spontaneous fluctuations in BOLD signal in the absence of any stimulus or task **frontiers:rsfc**. Resting-state functional connectivity measures the temporal correlation of spontaneous Blood Oxygen Level Dependent (BOLD) signals among spatially distributed brain regions, with the assumption that regions with correlated activity form functional networks. There are two methods that are most commonly used to examine resting state functional connectivity of brain networks, they are as follows:

1. Seed-based Correlation Analysis (SCA) and
2. Independent Components Analysis (ICA)

In seed-based approaches, activity is extracted from a specific region of interest and correlated with the rest of the brain. In contrast, ICA does not begin with pre-defined brain regions. It is a multivariate, data driven approach that deconstructs fMRI time-series data throughout the brain into separate spatially independent components **connectivityanalysis**. The resting-state fMRI study produces reliable and reproducible results, and several features of resting-state fMRI makes it favorable for investigating the functional correlation of various brain regions in psychiatric and neurological disorders. First, compared to the modular representations of traditional fMRI, functional connectivity provides a broader network representation of the functional architecture of the brain. Second, the absence of an explicit task eases the cognitive demand of the fMRI environment, thereby eliminating the problem of whether or not to match groups on task performance and allowing researchers to investigate under-studied populations, including infants and cognitively impaired individuals. Finally, the relatively standard manner in which resting-state fMRI data are acquired makes it ideal for multi-site investigations and data sharing **resting**.

2 Objectives

The objectives of the project are as follows:

2.1 General Objectives

- Perform analysis of the functional connectivity of the hippocampus in patients suffering from Major Depressive Disorder and acquire a comprehensive idea about how it compares to that of normal individuals of the same age group and sex.

2.2 Specific Objectives

- Deploy computational tools, and implement image processing algorithms for the exploration of MR image datasets of the human brain using AFNI.
- Explore data visualization tools, with emphasis on displaying functional brain networks.
- To perform Seed-based Analysis to explore functional connectivity within the brain based on the time series of a seed voxel or Region of Interest (ROI).

3 Problem Statement

3.1 Need For An Imaging Basis

Current diagnostic procedures involved in diagnosis of mental disorders do not have an imaging or a biochemical basis **nobiochemicalbasis noimagingbasis**. The diagnosis procedures that are the gold standard for the diagnosis of psychiatric disorders are wholly based on behavioral observations and patient reported symptoms. Two most widely established symptoms are used to classify these manifestations, one is the Diagnostic and Statistical Manual of Mental Disorders (DSM) and the other is International Classification for Diseases (ICD). Despite each being, widely used as the other, both of these diagnosis manuals are more like frameworks that provide a way of classifying a psychiatric disorder depending on patterns of behaviour rather than interpreting the etiology and the physical cause of those disorders. This statement alone raises an argument that "although reliable, current diagnostic procedures in psychiatry are not entirely valid".

Let us take an example of the diagnostic procedure involved in the diagnosis of Major Depressive Disorder. The DSM-V, published in 2013, is the most up-to-date manual and is based upon the work of expert study groups and makes use of large sets of data. According to the DSM-V, for a person to be classified as "suffering from Major Depressive Disorder", he/she must report with either depressed mood or anhedonia (inability to feel pleasure in normally pleasurable activities) along with four out of eight additional symptoms **diagnosticbrainimaging**. This makes it possible for two distinct individuals who do not share a single symptom in common and to receive similar treatment (or medication) for MDD. Furthermore, the current diagnostic procedures such as the DSM-V are not perfect. For example, impulsivity, emotional lability (the property of changing rapidly), and difficulty with concentration each occur in more than one disorder. Now, the fact that different exemplars of the same category can share no symptoms and that the exemplars of two different categories may share common symptoms, raises questions about the validity of the current diagnostic procedures in psychiatry. In addition to that, some other medical conditions such as thyroid disease, brain tumors, and vitamin deficiency can mimic depression-like symptoms **externalfactorsinMDD**. Therefore, a diagnosis may also have to be conducted in order to rule out some other medical condition that might be causing depressive symptoms. For instance, a blood test can be done to ensure the symptoms are not due to thyroid related issues.

3.2 Reverse Inference Fallacy

In the present day and world, a variety of imaging modalities such as ultrasonography, x-rays, computed tomography, MRI, SPECT, PET, fluoroscopy, etc are being implemented for a large number of purposes, most of them include clinical diagnosis of various diseases and the others include research. Now, while some of these imaging modalities such as MRI, SPECT and PET

are indeed being used for research that involves the diagnosis of psychiatric disorders, they are yet to be implemented for the actual diagnosis of mental disorders. There exist thousands of published research studies using functional neuroimaging methods such as SPECT, PET, and fMRI that revolve around diagnosis of mental disorders. In spite of that, the findings from these research do not appear amongst the diagnostic criteria; aside from its use to identify potential physical injury or tumours, neuroimaging is not used in diagnostic procedure in psychiatry.

The fact that advanced imaging techniques are not being used for diagnosis of mental disorders, especially after so many researchers have done studies on this regard, may seem a bit counter-intuitive at first but the arguments produced by reverse inference against it is quite sensible and valid. Reverse inference is a kind of reasoning that is applied to infer the involvement of a specific cognitive process from observed brain activation during a task. It attempts to uncover specific cognitive processes or behaviours that my be associated with specific structural or functional brain alterations.

However, reasoning backwards from brain activity is problematic because neurological disorders, such as MDD, are multifaceted and are influenced by several factors such as concurrent diseases, disease history and artifacts. For example, in the following figure we can see that a radiologist can assure that a person's arm is broken or not by taking a look at the radiograph. However, a radiologist cannot assure a patient is suffering from this particular mental disorder my taking a look at the CT scan of the patient's brain due to reverse inference fallacy.

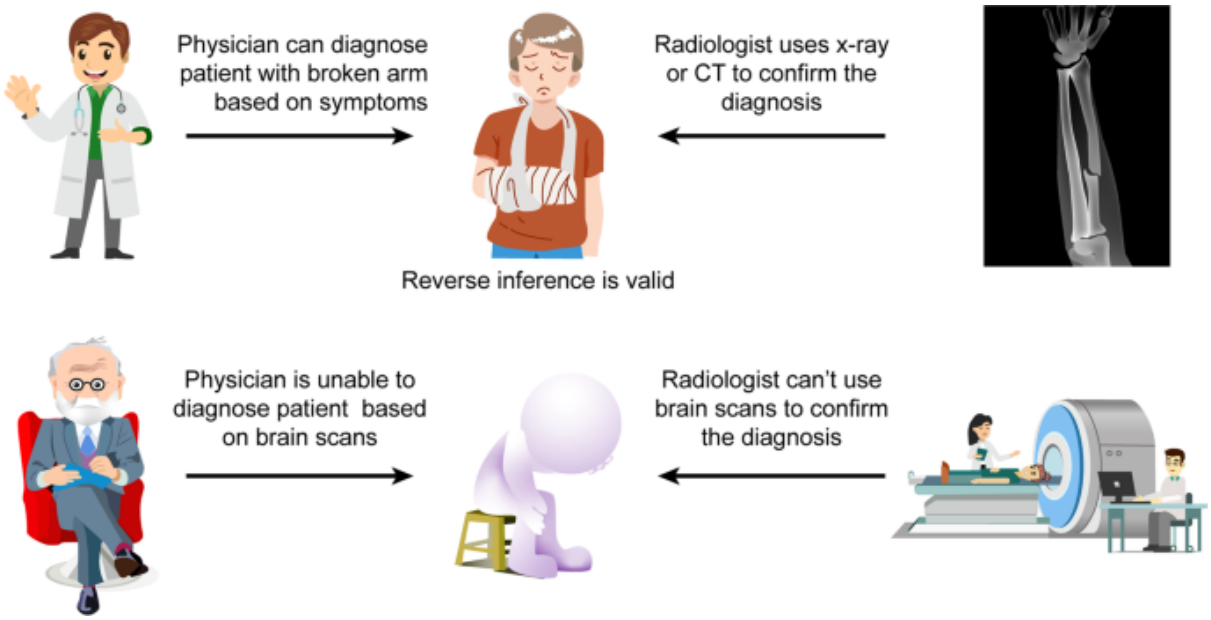

Figure 3: Illustration of reverse inference fallacy in diagnostic psychiatry

Most psychiatric imaging studies involve subjects from only two categories: patients from a single diagnostic category and people without any psychiatric diagnosis (healthy individuals), the most that can be learned from such a study is how brain activation in those with a particular disorder differs from brain activation in those without a disorder. This raises a dilemma for

the diagnosing clinician, as the question is not "does this person have disorder X or are they healthy?" but rather "does this patient have disorder X,Y,Z or are they healthy?" because the pattern of images that distinguishes patients with disorder X from healthy people may not be unique to X but shared with a other disorders.

In addition to the reverse inference fallacy, standardization is another issue that contributes to neuroimaging not yet finding a place in psychiatric practice. Standardization is relevant in the sense that protocols for imaging studies differ from study to study, particularly amongst functional imaging studies. Findings on the patterns of activation acquired in studies of psychiatric patients depend strongly on the task being performed by the subjects and the statistical comparisons made by the researcher afterwards. Such findings are pretty much incomplete unless they include the information about which task evoked the activation in question: whether the patient wa resting, processing emotional stimuli, resisting emotional stimuli or engaged in some other task? Therefore, the fact that the imaging study's conclusions are relative to the tasks performed adds further complexity to the problem of consistently discriminating patterns of activation of healthy and ill subjects.

Now, in the word of technology that is advancing day in day out, development of more sophisticated methods of image analysis may hold promise discerning the underlying differences among the many disorders that feature similar regional abnormalities. Nevertheless, the scope of neuroimaging seems promising for the aid in the diagnosis, and hopefully treatment of psychiatric disorders. Considering the above mentioned arguments, an imaging basis for the diagnosis of mental disorders seems like the need of the hour.

4 Review of Literature

4.1 Background

Efforts are continuously being made to discover reliable biomarkers for the clarification of biological mechanisms that are involved in psychiatric disorders, identification of subjects at risk and provide etiology-based treatments. Imaging modalities such as structural magnetic resonance imaging (sMRI) and functional magnetic resonance imaging (fMRI) are used to outline brain irregularities over Major depressive disorder (MDD)**structuralbrainimaging fMRIFuture**. Multiple imaging modalities have been considered for the assessment of functional connectivity of brain networks, but fMRI is the most commonly used amongst the others. This is mostly because fMRI imaging methods such as echo-planar sequences are sensitive to changes in the blood oxygenation level dependent (BOLD) signal that reflects neuronal activation **rogersassessing**. Most studies that have been referred to, whilst writing this review have utilized the fMR-imaging modality to conduct investigations on the core aspects of functional brain alterations in patients suffering from MDD. The goal of this literature review is to gain comprehensive knowledge about the associations of various brain networks with Major Depressive Disorder and also to acquire a brief overview of how the brain networks, especially the hippocampal network, gets affected by MDD. Since this project is mostly concerned with the hippocampal network, most of the literature will more or less be related to the temporal lobe and its structures.

4.2 Functional Magnetic Resonance Imaging for the Assessment of MDD

People with MDD show distinct structural and functional alterations that differ from those of healthy individuals. Unlike structural brain imaging that captures the anatomical structures present in the brain, functional brain imaging involves measurement of blood flow and metabolism to visualize the activation of specific brain regions. Nearly all fMRI studies utilize blood oxygenation level-dependent (BOLD) fMRI for assessing functional brain activity. A brain region becomes active when there is blood flow in that region, and an elevated level of metabolism. Brain activation results in increased consumption of oxygen, which increases the inflow of oxygenated blood and leads to an elevated BOLD signal. BOLD fMRI allows for regional and global mapping of brain regions activated during non-task (i.e. rest) and task-related activities **zhuo2019rise**. fMRI comes in two flavours, one is the resting-state fMRI and the other is the task-based fMRI. “Resting state” is when a person is fully awake but isn’t performing any particular stimulus or task that requires attention and cognition. While many studies are based on the rs-fMRI, researchers believe that the rs-fMRI lacks the linearity and stationary signals required for the assessment of MDD.

Several researches and evidence have found reduced hippocampal volumes in depressed patients compared with healthy controls. Videbech and Ravnkilde discovered that hippocampal volumes were 8–10% smaller in patients with MDD when compared with healthy controls **zhuo2019rise**. Likewise, Bremner et al. found that left hippocampal volumes were 19% smaller in MDD patients when compared with healthy controls**zhuo2019rise**. **zhuo2019rise**, in their paper have also showed functional alterations in the activity of the right hippocampus, right para-hippocampal gyrus, left amygdala and the entire caudate nucleus, which ultimately suggests that the temporal lobe and various structures in the temporal lobe, such as the hippocampus might have important pathophysiology of MDD. Therefore, additional studies are needed to determine the relevance of these findings.

In a task based fMRI study, Fu et. al. found that presentation of sad faces led to increased activation of the left hippocampus, mainly the amygdala and the parahippocampal gyrus **correlation**. It shows the evidence that MDD patients have a hard time recognizing and processing emotion in facial expression (sad vs. happy).

4.3 Resting State Functional Connectivity of Hippocampal Networks

There has been a plethora of studies that focus on the abnormal functional connectivity of several brain networks in patients with MDD. A lot of these studies show that MDD not only shows associations with regional deficits, but also with abnormal functional integration of distributed brain regions. Several brain regions with abnormal activities in the resting-state have been identified to be associated with MDD, such as the para-hippocampal gyrus, prefrontal cortex, cingulate gyrus, fusiform gyrus, and thalamus. Moreover, disruptions in functional connectivity have been observed between specific pairs of regions in MDD through functional connectivity analyses which may or may not include seed-based correlation analysis **homogeneity**.

frontiers:rsfc used the independent component approach (ICA), selecting a set of regions with shared fMRI signal fluctuations and a high degree of spatial similarity to the DMN, and reported increased connectivity with the thalamus and the subgenual ACC in depression. Many studies have found that the hippocampus, which is a complex structure embedded deep into the temporal lobe, plays an important role in MDD. Hippocampus can be subdivided into 3 sub-structures, and these structures are listed below:

1. Cornu Ammonis (CA)
2. Dentate Gyrus (DG)
3. Subiculum

Various studies have been performed with each of these sub-structures as the seed or the region of interest. **rightinsula** found increased connectivity in the left premotor cortex (PMC) and reduced connectivity in the right insula with the CA seed region. Similarly, increased connectivity was reported in the left orbitofrontal cortex (OFC) and left ventrolateral prefrontal cortex (vPFC) with the DG seed region. The subiculum seed region revealed increased con-

nectivity with the left premotor cortex (PMC), the right middle frontal gyrus (MFG), the left ventrolateral prefrontal cortex (vPFC) and reduced connectivity with the right insula. In addition to that, region-of-interest based correlation analyses performed on rs-fMRI data showed positive functional connectivity between the hippocampus in limbic system, subcortical areas, temporal lobe, medial and inferior prefrontal cortex, while at the same time, negative functional connectivity was in bilateral prefrontal cortex, parietal and occipital cortex and the cerebellum **cerebellum**. Furthermore, multiple research has implicated abnormalities in the prefrontal-hippocampal neural circuitry in patients suffering from MDD **prefrontal**. fMRI studies have also found abnormal hippocampal activation as well as abnormal functional connectivity of prefrontal-hippocampus circuitry in adults who were suffering from MDD. Moreover, Peng et. al. in one of their recent studies reported decreased rsFC between hippocampus and insula in medication-resistant adult patients **pengetal**.

The hippocampus has been proven to play an important role in memory **memory** and emotion processing **emotion**. Functional abnormalities of the hippocampus in adult MDD have been consistently reported in several fMRI studies. According to an fMRI study, decreased brain activity in the hippocampus was reported in depressive patients. The hippocampus and the amygdala of MDD patients showed an overlapping pattern of reduced FC to the dorsomedial-prefrontal cortex and fronto-insular operculum. Both of these regions are known to regulate the interactions among intrinsic networks (i.e., default mode, central executive, and salience networks) that are disrupted in MDD **disruptedMDD**. A few postmortem studies have found decreased cellular density in the hippocampus, including one study that showed patients with MDD have fewer anterior dentate gyrus granule cells than control subjects **controlsubject**. However, functional imaging studies at this resolution in patients with MDD are lacking.

For several reasons, researchers have focused on the role of the hippocampus in depression. The hippocampus is involved in the regulation of the hypothalamic pituitary adrenal (HPA)-axis, which is responsible for the production of stress-related glucocorticoids such as cortisol **cortisol**. In this context, depressed individuals have been found consistently to report high levels of stress **stress**, which is reflected biologically in elevated rates of hypercortisolemia and disturbed HPA-axis functioning. Moreover, depressed patients have also been found to be characterized by difficulties in hippocampal-dependent learning and memory **learning**. Also, Problems can occur when excessive amounts of cortisol are sent to the brain due to a stressful event or a chemical imbalance in the body.

4.4 Overview of Hippocampal Circuitry and its Functions

Hippocampus is a complex brain structure embedded deep into temporal lobe. It has a major role in learning and memory. Hippocampus can can be related to the functional requirement of episodic memory and more specifically to the storage and retrieval of memory **hippocampal**. The hippocampus is part of the hippocampal formation which includes the DG (Dentate Gyrus), the hippocampus proper, and the subiculum gyrus **limbic**. The hippocampal gyrus contains areas such as the EC (entorhinal cortex) and subiculum, which are both vital in the flow of information through the hippocampus. The following figure**hippocampalcircuitry** is a schematic representation of the hippocampal circuitry:

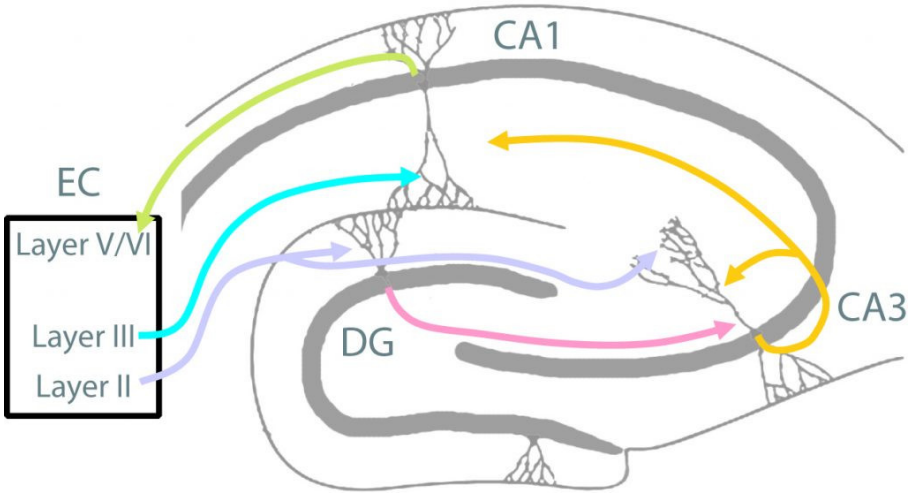

Figure 4: Schematic Representation of Hippocampal Circuitry

The neural circuit in the hippocampus is referred to as the hippocampal circuitry, or as the trisynaptic. The hippocampal circuit is divided into CA1, CA2, CA3, CA4, EC and the DG regions. These regions coordinate information from a variety of sources. Some inputs to the hippocampus arrive from the entorhinal cortex pass through to the DG. From the DG, connections are made to CA3 of the hippocampus via mossy fibers and then to CA1 via Schaffer collaterals. From these two CA fields information then passes through the subiculum entering the alveus, fimbira and fornix and then to other areas of the brain.

Information flows into and through the hippocampus via. three principal pathways **limbicsystem:hippocampu**

1. The perforant pathway: From the entorhinal cortex to granule cells of the DG.
2. The mossy fiber pathway: From the granule cell of the dentate gyrus to the pyramidal cells of the CA3 region of the hippocampus.
3. The Schaffer collateral pathway: From the CA3 region to the CA1 region of the hippocampus.

The CA1 region has an important role in memory due to the high levels of NMDA glutamatergic receptors located in the CA1 Schaffer collateral neurons. The synaptic plasticity of these neurons heavily relies on long-term potentiation (LTP) induction to allow the strengthening of declarative memory which includes two types of implicit memory: episodic and semantic memory such as facts and events. CA2 is a small region located between CA1 and CA3. It is often ignored due to its small size. The CA3 is a portion of the hippocampal formation adjacent to the dentate gyrus. **hippocampalca3** have found that the CA3 plays an essential role in the consolidation of memories when examining CA3 regions using the Morris water maze.

The entorhinal cortex (EC) is a structure in the brain located in the medial temporal lobe. The EC is composed of six distinct layers. The superficial (outer) layers, which includes layers I through III, are mainly input layers that receive signals from other parts of the EC. The deep (inner) layers, layers IV to VI, are output layers, and send signals to different parts of the EC and the brain. Layers II and III project to the CA3 area of the hippocampal formation (via the perforant path) and to the granule cells of the dentate gyrus, respectively. The dentate gyrus (DG) is the innermost section of the hippocampal formation. The dentate gyrus consists of three layers: molecular, granular, and polymorphic.The granule cells (present in granular layer) are the major source of input of the hippocampal formation, receiving most of their information from layer II of the entorhinal cortex, via the perforant pathway. Information from the DG is directed to the pyramidal cells of CA3 through mossy fibres.

4.5 Important FCs for Diagnosis of MDD

Default mode network (DMN), Anterior salience network (ASN) and Executive control network (ECN) are the brain networks linked with clinical depression. Increased functional connectivity within the DMN is primarily associated with depression. At the same time, it is found that the DMN-ECN and the DMN ASN pairs have fewer interactions or connectivity during episodes of depression. Within the ECN, the functional connectivity may be excessive or deficient. Furthermore, the ECN in depressed women is correlated with negative self-directed thoughts and the ECN-DMN functional connectivity is related to rumination. Researches have shown that ASN which includes main emotional areas maybe over, under or normally connected in depression. Depression is also related with the impaired functional connectivity of other brain networks besides the ones mentioned above. In addition to that, the Posterior Cingulate Cortex, which is a part of the DMN has shown significant relationship with the hippocampal network, albeit this is not just specific to MDD.

LRbrainnetwork, on comparing the functional activity within DMN, found that there was decreased functional connectivity within DMN network in depressed people, which contradicted most research that have been conducted. This may be because the patients involved in their study had mild to moderate depression which showed similar cognitive features as in depression (MDD) like rumination and control deficits, but lacked neural markers present in typical serious condition like MDD. Many studies that have been published showed increased func-

tional connectivity within DMN in depression, which was found to be contradiction with one particular research which may be due to sample differences like severity of the disorder, age group, and other demographics.

Significant rs-fMRI differences between groups were identified in multiple clusters in the DMN and ECN. Greater positive connectivity within the ECN and between ECN and DMN regions was associated with poorer episodic memory performance in the group of healthy individuals but better performance in the MDD group. Greater connectivity within the DMN was associated with better episodic and working memory performance in the Non-Depressed group but worse performance in the MDD group. These results provide evidence that cognitive performance in MDD may be associated with aberrant functional connectivity in cognitive brain networks and suggest patterns of alternate brain function that may support cognitive processes in MDD. Results also showed that the DMN–left fronto-parietal network is the pair discriminating between healthy and depressed people to the highest degree. According to Davidson and Heller models, left prefrontal activity is related to positive emotions and motivations, while right corresponds to negative emotions and withdrawal of motivation **model**. Relatively active right prefrontal area and idling left prefrontal cortex together may be a neurophysiological signature of depression. Therefore, coupling of left prefrontal with DMN denotes its passivity and that there is less approach motivation, less happy mood which is one of the most important depression related signs. Increased functional connectivity between left fronto-parietal network and subsystems of the DMN can be seen in fMR images of depressed patient.

4.6 Structural Changes associated with MDD

The latest research **brainvolume** shows that the size of specific brain regions can decrease in people who experience depression. Researchers continue to debate which regions of the brain can shrink due to depression and by how much. Hippocampus, thalamus, amygdala and the frontal and prefrontal cortices are the regions that have reported the most shrinkage. The amount by which these areas shrink is linked to the severity and the length of the depressive episodes. In the hippocampus, for example, noticeable changes can occur anywhere from 8 months to a year. **effectMDD**

brainshrink, in her newspaper article stated that people experiencing their first depressive episode had a normal hippocampus size but as the number of episodes of depression a person had would increase, the greater the reduction in hippocampus size. It has been widely reported that there is a significant reduction in hippocampal volume in depression patients. This situation was found in both adult and adolescent depressed patients, whether they were in their first or recurrent depressive episodes. A recent study reported that, in female patients with recurrent familial pure depressive disorder (rFPDD), volumetric reductions of the right hippocampal body and tail were significantly larger than those of the left, while the whole brain volume was approximately equal to that of healthy subjects. There is evidence that stress via the hypothalamic pituitary-adrenal axis can result in elevated glucocorticoid levels in patients with

depression and can act on the glucocorticoid receptors in the hippocampus. Thus, hippocampal atrophy occurs as a result. Reduced gray matter volume and reduced functional activity in the hippocampus would lead to negative emotion and the inability of cognitive processing in depressive patients. Depression can also decrease neuronal dendrite branching and plasticity in the hippocampus.

In addition, depression can trigger activation of the hypothalamic-pituitary-adrenal axis, increase the level of corticosteroids, and down regulate hippocampal neurogenesis. Depression makes changes in hippocampal volumetric changes, hippocampal neurogenesis, and apoptosis of hippocampal neurons **effectMDD3**.

4.7 Reverse Inference Fallacy in MDD

The diagnosis of MDD relies heavily on patients for symptom recall. Like in many other mental disorders there is no specific physical symptom in MDD, therefore neurologists are unable to deduce MDD based on conventional MRI. Moreover, different mental disorders can produce similar structural or functional brain alterations which puts the neurologist in a much greater dilemma as they are unable to determine whether a specific alteration in the brain is attributed to MDD-alone. So, the reverse inference is invalid as structural or functional activity patterns from MRI cannot be used to diagnose a patient with a specific neurological disorder. Researchers, over the past few decades have been attempting to develop a "bridge", such as novel biomaterial, high resolution and multi-modality imaging technique, artificial intelligence, novel nanomaterials and quantitative electrical signal acquisition technologies to overcome the reverse inference fallacy that hinders the study of MDD. **zhuo2019rise**

Conclusion of the Literature Review

Evidence shows that major depressive disorder (MDD) patients at resting-state brain connectivities are aberrant compared with healthy controls (HC). Abnormal resting-state functional connectivities of distributed brain networks are believed to contribute to the MDD illness process. Reliable, reproducible and valid conclusions must be derived from these types of studies, for imaging modalities such as fMRI to not only aid in the diagnosis but also to optimize patient care, reduce treatment resistance and shorten the duration of illness.

After a thorough review of the literature, fMRI looks promising for providing excellent and reliable indexes for the aid in the diagnosis and ultimately treatment of MDD. Once it overcomes the aforementioned hurdles, fMRI imaging technique could become a clinical decision support tool that might reduce unsuccessful treatments and improve treatment efficacy and efficiency of mental disorders all together.

5 Feasibility Study

The following points addresses the feasibility of the proposed study:

- This project will primarily focus on analysis of the functional connectivity of the hippocampal network of patients suffering from Major Depressive Disorder.
- Although the approach of neurological study is new in Nepal, thousands of research has been conducted worldwide that involves similar approaches for the exploration of functional connectivity.
- Publicly available dataset will be used in this project. The MR images of MDD patients as well as that of healthy individuals for this project are retrieved form the DecNef Project Brain Data Repository. Data used in the preparation of this work were obtained from DecNef Project Brain Data Repository gathered by a consortium as part of the Japanese Strategic Research Program for the Promotion of Brain Science (SRPBS) supported by the Japanese Advanced Research and Development Programs for Medical Innovation (AMED). **dataset**
- There are not many materials or components required for the project. We will be using open-source softwares such as Analysis of Functional Neuro Images (AFNI) and the Linux operating system, this adds to the feasibility of this project.
- Furthermore, all the work involved in this project can pretty much be conducted virtually at home, therefore this eliminates the obstacles that may arise due to the on-going pandemic.
- Hence, the feasibility study to conduct this proposed project is positive and supportive.

6 Methodology

6.1 Methods and Materials

Functional Magnetic Resonance Imaging is well established as a method for the detection and delineation of regions of the brain that change their level of activation in response to specific conditions. fMRI studies, such as the one we will be conducting, implement imaging methods that are sensitive to fluctuations in the BOLD (Blood Oxygen Level Dependent) signal that reflects neuronal activation. Therefore, our methodology will approach the study of functional connectivity using BOLD signals, and since we will will perform seed-based analysis, these BOLD signals will be restricted to only a specific region of interest. We will adopt seed-based resting stat fMRI connectivity analysis in order to study alternations in functional connectivity of hippocampus region of fifteen volunteers suffering from major depressive disorder and compare the results with that of fifteen well-matched healthy controls. In addition to that, we will also attempt to explore how the changes in functional connectivity of MDD patients affect their emotional behaviour and memory.

We will use AFNI (Analysis of Functional NeuroImages). AFNI is one of the leading software suite of C, Python and R programs and shell scripts primarily developed for processing, analyzing and visualization of three dimensional human brain functional magnetic resonance imaging results. AFNI has a rich software package for processing and displaying fMRI data. In addition to that, several statistical analysis methods for 3D functional datasets are also available in this software. AFNI is an open-source software that was developed for reasearch purposes, however it is not cross-platform and only runs on UNIX based operating systems such as MacOS and Linux based systems like Ubuntu, Fedora, Linux mint, CentOS and Arch Linux. We will be running AFNI on Linux based systems.

6.2 Seed Based Functional Connectivity Analysis

Seed-based functional connectivity, also referred to as Region of Interest-based functional connectivity, finds regions that correlate with the activity in the seed region. In seed-based analysis, the correlation is compared between the time-series of the seed or the ROI and the rest of the brain. The correlation between different brain regions indicate that they are involved in the same underlying functional process and thus are interpreted as functionally connected. “Functionally connected” does not necessarily mean that these brain regions are directly connected by neuronal fibers. The overall connectivity of the brain will be visualized using AFNI. The main advantage of seed-based analysis is that the computation is simple and the interpretation of the results is intuitive. But as the seed region changes, the results of the functional connectivity will also change which is this method’s demerit.

6.3 Region of Interest Selection

For our study, we have chosen the hippocampus as the seed region or the region of interest. The reason for which we have chosen the hippocampus as our region of interest is three folds, one, since we are using publicly available dataset we had to settle for a suitable data that the repository had. Two, although there have been many studies about the functional connectivity other brain regions, there have been limited reports of functional alterations in the temporal lobe with the use of rs-fMRI imaging modality. The third reason for which we have chosen hippocampus to be our reason of interest is, MDD being associated with alterations in regional brain volumes, particularly hippocampus along with the functional changes in brain circuits makes it more convenient to ponder upon the hippocampal region of brain as the ROI. Therefore, our study will be centered towards the hippocampal region of the limbic system.

6.4 fMRI Data Acquisition

As mentioned earlier, we will be using the **dataset** public repository to acquire the fMRI images of patients diagnosed with major depressive disorder, as well as those of healthy controls. The brain imaging dataset that we plan to use for this project initially will have 3T MRI images of 1410 participants that were collected at 11 different sites. However, we will only be using a tiny fraction of this big data. Statistical approaches will be undertaken (statistical tests will be performed) in order to narrow down this big data to just 30 subjects.

The *SRPBS Multidisorder MRI Dataset* public repository has categorized the data set into two groups, healthy controls (HC) and depressed patients (MDD). There are more than 1410 subjects in this repository out of which only fifteen right handed volunteers from each category, the MDD patients and the HC, will be chosen. For the volunteers form the depressed category, only subjects whose BDI was greater than 30 will be selected. Since we will be acquiring the data from large scale public dataset, diagnosed by neurologist, this assures our research to be tilted more to accuracy and efficiency. All the participants, extracted from the public data set, have underwent a standardized clinical evaluation protocol, which included a general and neurological examination, which will make our research more feasible. Once selection of volunteers is complete, we will proceed to match both groups according to their age and sex. Since sex is a categorical variable, chi square test will be performed to measure the appropriateness of fit. For matching the age, t-test will be used to calculate the p-value.

The authenticity of the data acquired from the above mentioned dataset is guaranteed as the participants from the depressed category had undergone a standardized clinical evaluation protocol, which included a general as well as a neurological examination performed by licensed neurologists.

6.5 Image Pre-processing

Image pre-processing is the most important aspect of our project. Pre-processing is an important step which converts the raw fMRI data into a form that is understandable by each software package. Pre-processing is also important to improve the signal quality of the raw data obtained from the MRI scanner. Image pre-processing will involve steps like artifact detection, baseline correction, realignment, movement correction, co-registration, normalization, and smoothing. The fMRI data will be preprocessed using AFNI **coxafni**. Various different toolboxes will be used for fMRI preprocessing which will involve spatial or temporal filtering of the fMRI data and improving the image resolution. In addition to these steps, motion correction will also be done in order to minimize of the noise produced in the image due to artifacts. Seed based resting-state functional connectivity (rsFC) analysis of hippocampal network will be performed such that for each seed region, rsFC will be calculated as the correlation between its mean time course and the time course of every voxel in the brain. Seed-voxel correlation mapping is one of the simplest techniques for studying functional connectivity: the correlation coefficient between the fMRI signal at different times and measurements of activation in the seed region will be calculated separately for each voxel in the brain and will be displayed a parametric image. The final rsFC results, between the MDD patients and HC will be compared using statistical approach.

6.6 Statistical Analysis of Functional Connectivity

Once the rs-fMR images from the public data set has been pre-processed, statistical tests will be implemented to for a thorough analysis of the functional connectivity of the seed. Specifically, we plan to assess functional connectivity between various regions of the brain and hippocampal area. To assess functional connectivity in the brain region, Resting-state analyses, that is, time series correlations in BOLD fMRI data acquired in a task-free state will be used. A statistical approach to image analysis makes it possible to discover spatial and temporal patterns that correspond to the performance of specific tasks and specific diagnoses. Such statistical methods have only begun to be applied to clinical disorders but show promise for increasing the “specificity” of brain imaging markers for mental illness.

7 Cost Estimations

We have chosen AFNI (Analysis of Functional Neuro Images) in order to conduct this project. AFNI is a software, primarily developed for the analysis and display of multiple MRI modalities such as fMRI images. AFNI is an open-source software which runs on UNIX based operating systems such as macOS or Linux. Due to the high price of a computer from Apple Inc., we have chosen to run AFNI on top of a Linux operating system. Since, both AFNI and the Linux operating systems are free and open-source softwares, the cost of this project becomes extremely low. In addition to using free softwares, the data that we plan to use for this project is also available freely from the **dataset** public repository which further cuts down the cost of this project.

Nevertheless, we will require an SSD (Solid State Drive) on top of which we will install the Linux operating system and an external HDD for storing the data acquired from the public repository, the external HDD will allow us to easily carry and move the data amongst each other. Furthermore, if the findings from our project seems to be beneficial to the field of diagnostic psychiatry, we expect financial aid from the university concerning the release of our findings for academic journals or research papers.

The budget plan for this project is tabulated below:

S.N.	Cost Element	Cost Remarks	Rate (NRs.)	Quantity (Unit)	Total Cost (NRs.)
1	SANDISK SATA SSD (512 GB)	Fixed	8,500	4	34,000
2	External HDD (1 TB)	Fixed	4,000	1	4,000
3	Miscellaneous	Variable	-	-	7,000
4	Grand Total				45,000

Table 1: Cost Estimations

So, the total cost estimation **ssdprice** of this project is *NRs. 45,000 only*.

8 Proposed Work Flow

The following chart represents our plans regarding how we wish to proceed in this project.

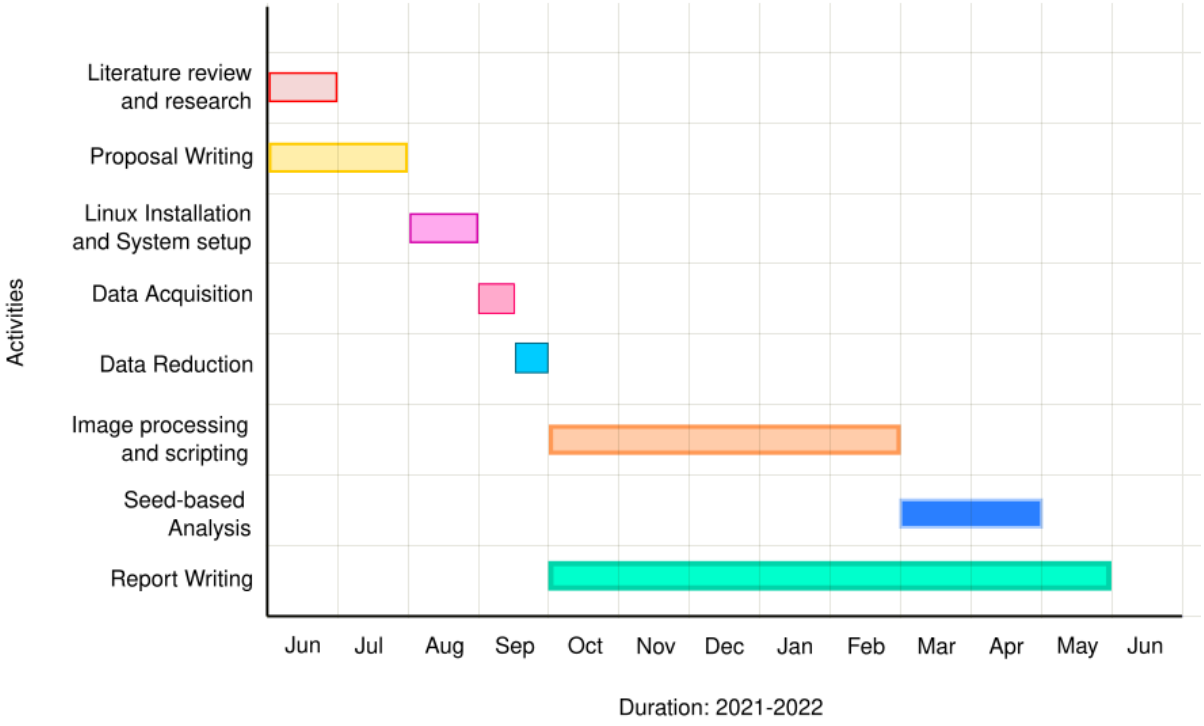

Figure 5: Gantt Chart illustrating the proposed workflow

9 Conclusion

Previous studies indicated discrepant functional connectivities between MDD patients and HC. However, it is unknown whether these connectivities can be used as diagnostic biomarkers of MDD.18 Indeed, whether the future diagnostic models built on the functional connectivity values can improve treatment prediction and clinical outcome depend on its accuracy performance. While the results might not be sufficient to provide a detailed understanding of the complex and changing functional connectivity of the brain which could make actual diagnosis of MDD, this project can lay the foundations for further research and development.

References