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Progress Report

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.

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Abstract

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Abbreviations

AFNI Analysis of Functional Neuroimages

BDI Beck Depression Index

BOLD Blood Oxygen Level Dependent

CSF Cerebrospinal Fluid

fMRI Functional Magnetic Resonance Imaging

GM Gray Matter

HCs Healthy Controls

MDD Major Depressive Disorder

MR Magnetic Resonance

rs Resting State

rsFC Resting-state Functional Connectivity

SCA Seed-based Correlation Analysis

sMRI Structural Magnetic Resonance Imaging

SPM Statistical Parametric Mapping

WM White Matter

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1 Introduction

So, based on our objective proposed, this refers to the progress report of our project till date along with the future works to be performed that completely meet our aims.

Functional MRI 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 stimulus. fMR imaging modality is sensitive to fluctuations in the BOLD signal which reflects neuronal activation, or neuronal activity.

1.1 Background

The specific objective of this project is to perform seed-based functional connectivity analysis to study how the normal function of a particular area of a healthy brain gets disrupted in diseased conditions. Major Depressive Disorder being one of the major mental health illnesses in our country, this project aims to analyze the functional changes in the brain of MDD patients compared to healthy controls. Furthermore, in our literature review, it was found that the hippocampus of the brain is one of the major regions affected by a variety of neurodegenerative and mental disorders. For this reason, we aim to assess the functional connectivity of the hippocampal region of the brain.

1.2 Rationale

The absence of biological markers makes it exceptionally difficult for neurologists to diagnose a person with a psychiatric disorder. The diagnostic procedures that are the gold standard for the diagnosis of neurodegenerative and psychiatric disorders, in the present day, are wholly based on behavioral observations and patient reported symptoms, both of which do not have a molecular or radiological basis. Although there have been countless studies conceptualizing the possibility of implementation of various functional imaging modalities for deciphering the etiology and the functional effects of various mental disorders, the findings from these studies do not appear amongst the diagnostic criteria. A critical barrier to the clinical translation of many such findings is the reverse inference fallacy.

The information from structural radiology modalities describe the shape, size and integrity of brain structure, but they do not provide any information about the brain function. Nonetheless, as we will discuss in the coming sections of this progress report, combining structural MRI and functional MRI can be a promising technique to characterize normal and abnormal brain function, which can act as an auspicious biomarker for neurodegenerative or psychiatric disorders to determine the risk, progression and therapeutic effectiveness. This project can lay the foundations for further research and development in this particular field.

2 Methodology and Review on Individual Tasks

2.1 System Setup

For the following project, the primary software tool that we will use for functional connectivity analysis is AFNI. AFNI (Analysis of Functional NeuroImages) is an open-source software, distributed freely under the GNU General Public License. AFNI is used for processing, analyzing and displaying several MRI modalities such as anatomical MRI, functional MRI (FMRI) and diffusion weighted (DW) data. AFNI runs virtually on any UNIX based system such as macOS and GNU Linux. For financial reasons, we opted to install GNU Linux. Along with AFNI, we will also use SPM (Statistical Parametric Mapping) which is an image processing package in GNU Octave. GNU Octave, and all of its associated programs, is freely distributed under the terms of the GNU General Public License.

GNU Linux is an open-source Unix-like operating system. The Linux kernel is licensed under the terms of GNU General Public License version 2 (GPL-2.0). Popular Linux distributions include Ubuntu, Linux Mint, Fedora, and Arch. After we collected the SSDs that we requested for, we installed various linux distributions, namely Ubuntu, Linux Mint and Arch Linux. After installation of Linux, we installed AFNI and other related software on our Linux systems.

2.1.1 Runtime Environment

Here is a list of the major softwares used, along with their version information, during the runtime for the codes and results included in the following report:

- Operating System: Arch Linux x86_64, kernel version 5.16.8-arch1-1
- AFNI, version AFNI_22.0.03 'Hadrian'
- GNU Octave, version 6.4.0

2.2 Data Acquisition

The functional and anatomical MR images for the progression of this study were acquired from the SRPBS Multidisorder MRI Dataset. The datasets included were obtained from the DecNef Project Brain Data Repository, which is a repository of neurological images gathered by a consortium as a part of the Japanese Strategic Research Program for the Promotion of Brain Science supported by the Japanese Advanced Research and Development Programs for Medical Innovation (AMED). Furthermore, the MR datasets included in this repository were diagnosed by trained and experienced neurologists, which assures that our study will be tilted more towards accuracy and efficiency [1]. We acquired functional MR as well as T1 weighted images of subjects from two distinct categories. The first set of subjects are categorized as healthy controls, and the second set of subjects are categorized as those who are suffering from major depression. The subjects from the first category, i.e. healthy controls will be referred to as "HC" and the subjects from the second category, i.e. depressed subjects will be referred to as "MDD patients".

2.3 Data Selection

The SRBPS dataset originally had the MR images of more than 1400 volunteers. All of these volunteers had undergone a standardized clinical evaluation protocol, which involved a general and neurological evaluation. This opts for the accuracy of our study.

The following is a brief summary of the tasks performed during this step:

Selection of Data	
Status during the reporting period	Completed
Actions	Out of the data of 1400 volunteers from the SRBPS dataset, 15 healthy controls (diagnosis 0) and 15 depressed patients (diagnosis 2) were manually selected by just eyeballing the data sheet included in the data acquired from the SRBPS public repository.
Decisions	• The datasheet contained data of volunteers from various different site. Subjects of either sex, male or female, were selected from only one specific site, HUH in particular. The reason behind this was to make sure that the images that we will be working on were acquired from the same MRI scanner. This ensure consistency of data. • Majorly depressed patients were labeled with a BDI (Beck Depression Index) greater than 30. So subjects having BDI > 30 were only selected. • Trying to acquire data of people from a certain age group was a failure because there was not just enough data of the volunteers with BDI > 30. So we had to settle with subjects of the age group 20 to 50.
Testing & Verification	Once we had the subjects decided, two statistical tests were performed: 1. Chi-square test 2. t-test Chi-square test was performed to check the goodness of fit between healthy controls and depressed subjects based on sex and the t-test was performed to check the goodness of fit based on age.

The results of both t-test and the chi-square tests were positive (Appendix A), therefore we concluded that the subjects we selected for both categories, HC and MDD were well matched.

2.4 Data Preparation

After we finalized the subjects on which we will be working on, the image data that we acquired were converted into appropriate formats that could be used for further processing. The original image data that we acquired were in DICOM image format.

Conversion of image data from DICOM format to NIfTI format	
Status during the reporting period	Completed
Actions	Created a BASH script to add the NIfTI extension i.e. ".nii" in order to convert the image data of 15 HC as well as 15 MDD patients from DICOM format to NIfTI format. (Appendix B)
Decisions	• A DICOM file is an image saved in Digital Imaging and Communication in Medicine (DICOM) format. DICOM format is an internationally accepted format in which medical images are scanned, stored, retrieved and shared. • However, different scanner manufacturers extend the DICOM format in a variety of ways, which often results in duplication of information and incompatibilities between softwares. The Neuroimaging Informatics Technology Initiative (NIfTI) file format has become the standard for neuroimaging studies. In addition to that, the software tools (AFNI and SPM) that we will be using for image processing, analysis and visualization require images to be stored in the NIfTI file format. For these reasons, the original datasets which were in the DICOM format were converted into NIfTI format.
Testing & Verification	The images after conversion to NIfTI were opened in AFNI to verify that it could understand and render the image data in its interface.

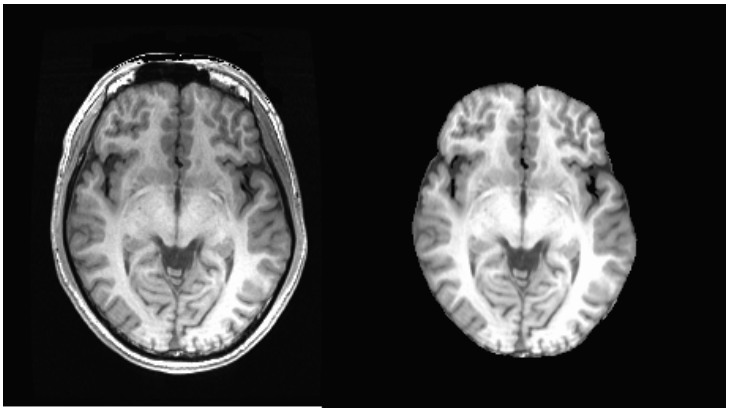
Furthermore, once the image data were converted into the NIfTI format, which could be understood by a variety of software tools that we use, the multiple 3D rsfMRI image data was converted into a single 4D BOLD data for each subject from either category.

Conversion of 3D fMRI image into 4D BOLD Data	
Status during the reporting period	Completed
Actions	A file conversion tool called dcm2nii with a graphical user interface was used to select all the 3D rsfMRI volumes to create a compressed file format containing the 4D BOLD data.
Decisions	• Unlike an anatomical MR image which is a single volume, a functional MR image is acquired in blocks, where each block represents the functional MR signal acquired at a given time. It is essential to merge these multiple 3D functional MR image volumes into a single 4D functional MR image, where time is the fourth dimension.
Testing & Verification	This step didn't require any testing. Nonetheless, the compressed file was decompressed and its size was checked to verify it was greater than 0 bytes.

After these two steps, our image data for both HCs as well as MDD patients were prepared for further processing.

2.5 Skull Stripping

Since high resolution structural images contain considerable amounts of non-brain tissue such as eyeballs, bone, skin, amongst other tissues. Skull stripping improves the quality and accuracy of the normalization and templates that will be created for skull-stripped images. The skull stripping was achieved in AFNI. The skull stripping process completes in the following steps:

<table>
<tr><td colspan="2">Extraction of the brain tissue</td></tr>
<tr><td>Status during the reporting period</td><td>Completed</td></tr>
<tr><td>Actions</td><td>AFNI provides a skull stripping tool called 3dSkullStrip. A shell script was created to implement this 3dSkullStrip program to extract the brain tissues in T1 weighted images of each subject from either category. (Appendix C)</td></tr>
<tr><td>Decisions</td><td>• After data preparation, our next step would be segmentation of various brain structures from the brain. But before doing just that, the brain tissues must be extracted by removing the surrounding skull in order to isolate the brain tissue from non-brain tissue from an MRI image of a brain.
• We decided that it would be better to strip the skull from T1 images in order to exclude gross spatial image non-uniformity artifacts and to reposition the brain in a reasonable manner.</td></tr>
<tr><td>Testing & Verification</td><td>The generated files were visualized in the AFNI interface. The following figures represent a basic visualization of the results for an axial image with z at 0.404 mm.
 
Figure 1: Axial Brain Image with Skull and without skull</td></tr>
</table>

Once the brain tissue is extracted from the T1 image, we proceed to image segmentation.

2.6 Image Segmentation

Image segmentation is the process of partitioning a digital image into multiple image segments, or image regions which essentially are a similar set of pixels. The primary goal of image segmentation is feature extraction, to simplify and change the representation of an image into something that is more meaningful and easier to analyze. Most, if not all, of the analysis of medical images requires some form of segmentation or feature extraction. Segmentation makes it easier to analyze any given image, as it distinguishes structures, regions or tissue classes of interest from other details in the image. Segmentation depends on a variety of features that are contained in the image. The features can be either color or texture or something else. The primary reason for image segmentation for our purposes is to reduce the information for easy analysis.

For our purposes, the image segmentation part involves segmentation of the gray-matter, white-matter and the cerebrospinal fluid in the T1 weighted image. The segmentation is carried out using SPM12 in GNU Octave. SPM (Statistical Parametric Mapping) is an image processing package of octave which requires the images to be in the NIfTI file format. SPM12 employs an algorithm that performs segmentation by characterizing intensity distributions of different tissue classes. Gray-matter contains high densities of unmyelinated (lacking a myelin sheath) neurons, white-matter contains high densities of myelinated neurons and CSF contains an ultrafiltrate of plasma and protein. This renders the intensities of these tissues differently in the image. SPM uses region based segmentation. Region based segmentation essentially extracts different regions of the brain into separate files. Region based segmentation further includes:

- Threshold Segmentation:

  In threshold segmentation, the image grayscale information processing is directly divided based on the gray values of various targets. Segmentation effect can be obtained if the target and background have high contrast. Threshold detection can be employed either locally or globally on the entire image. Local thresholding involves selecting variable segmentation threshold for different regions of the image based on the target regions and backgrounds. Global thresholding on the other hand only uses a single threshold for the entire image.

- Regional Growth segmentation:

  In regional growth segmentation, a particular seed pixel is selected and an intensity uniformity constraint is set. Then all voxels around the seed are examined to see if their intensities are sufficiently similar to those already in the region. Those pixels that satisfy the uniformity constraint are added or merged around the seed pixel.

In an ideal case, the files generated at the end of the segmentation are meaningful and contain a distinct set of pixels that represent a distinct brain region. In MR imaging system, the inherent magnetic inhomogeneities may cause a variation in intensity of a particular tissue

across the field of view. In addition to that, the intensity of a single voxel may be composed of signal from more than one tissue type. This is called partial volume effect. This partial volume effect has consequences in classification of the brain-tissue into gray-matter, white-matter and CSF in T1-weighted images. Partial volume effects between white-matter (bright) and CSF (dark) result in voxels with an inbetween intensity which can be misclassified as gray matter. Here is a brief overview of the tasks performed and their description during image segmentation:

Segmentation of Gray matter, White matter and CSF	
Status during the reporting period	Completed. (The results of segmentation are improper for certain subjects so this needs to be redone)
Actions	Used the interactive GUI of SPM12 to automatically identify and segment different tissue types within the images. A batch of T1 scans to be segmented were specified, along with a list of tissues that were to be identified.
Decisions	• Segmentation was only performed for T1-weighted images of healthy controls. • The version of GNU Octave that we used for segmentation had a slight bug which needed manual intervention before the images could be segmented. The source code for the segmentation script had to be modified. • Some members of the team opted for an older version of Octave.
Testing & Verification	SPM produces several files with the segmented data. Files beginning with "c1" is what the algorithm identifies as the gray matter; files beginning with "c2" is what the algorithm identifies as white matter and files beginning with "c3" is what the algorithm identifies as the CSF. Each of these files were visualized for all subjects in AFNI to verify that segmentation was performed correctly.

The output of the segmentation will be used for achieving a more accurate inter-subject alignment using DARTEL. The segmentation image data will also be used to generate a common mask.

2.6.1 Template Creation using DARTEL

DARTEL (Diffeomorphic Anatomical Registration Through Exponentiated Lie Algebra) is a toolbox available in SPM. DARTEL can be used to create templates that have a more accurate inter-subject alignment of various tissues. This is achieved by modeling the shape of each brain using millions of parameters. DARTEL achieves accuracy of such proportions by generating its own increasingly crisp average template data, to which the data are iteratively aligned. Each iteration makes individual images fit each other more precisely.

We need DARTEL because it allows an accurate inter-subject registration of brain images which is necessary for both proper separation of different segment, different tissue classes and after it gets specially normalized towards common global MNI we will be able to do group wise statistics and to extrapolate those findings to result of other studies.

Processing begins with the "import" step. This involves taking the parameter files produced by the segmentation, and writing out rigidly transformed versions of the tissue class images, such that they are in as close alignment as possible with the tissue probability maps. The next step is the registration itself. This procedure begins by creating a mean of all the images, which is used as an initial template. Deformations from this template to each of the individual images are computed, and the template is then re-generated by applying the inverses of the deformations to the images and averaging. This procedure is repeated a number of times. After running DARTEL by selecting the previously generated c1, c2, c3 images of all the fMRI images of HC,the file names beginning with "r" (as in "rc1") are the DARTEL imported versions of the tissue class images, which will be aligned together next.

Segmentation of Gray matter, White matter and CSF	
Status during the reporting period	Completed. (The results of segmentation are improper for certain subjects so this needs to be redone)
Actions	Used the interactive GUI of SPM12 to automatically identify and segment different tissue types within the images. A batch of T1 scans to be segmented were specified, along with a list of tissues that were to be identified.
Decisions	• Segmentation was only performed for T1-weighted images of healthy controls. • The version of GNU Octave that we used for segmentation had a slight bug which needed manual intervention before the images could be segmented. The source code for the segmentation script had to be modified. • Some members of the team opted for an older version of Octave.
Testing & Verification	SPM produces several files with the segmented data. Files beginning with "c1" is what the algorithm identifies as the gray matter; files beginning with "c2" is what the algorithm identifies as white matter and files beginning with "c3" is what the algorithm identifies as the CSF. Each of these files were visualized for all subjects in AFNI to verify that segmentation was performed correctly.

2.7 Image Preprocessing

The image preprocessing is entirely done with AFNI. The following image preprocessing steps were accomplished:

2.7.1 Preprocessing of BOLD fMR Images

The preprocessing of an fMRI image is basically done to improve the quality of the image so as to analyse it in a better way. Preprocessed images can suppress undesired distortions and enhance some features which are necessary for the particular purpose we are working towards. An fMRI volume contains not only the signal that we are interested in, which are the changes in oxygen levels of blood flowing to a certain brain region, but also fluctuations in the signal that can be caused by a variety of reasons such as involuntary or voluntary movement of subjects during the scan, random drifts, breathing artifacts, and heartbeats. These fluctuation in the signal need to completely reduced if possible otherwise they need to be reduced as much as possible.

The image resolution of a raw fMRI image is extremely low. The fMR signals are incredibly faint and are very brief, due to which they need to be acquired extremely fast before the signal disappears. This is the primary cause of low resolution of fMR images. Preprocessing of BOLD fMR images include reconstruction of the image, along with improving the quality of the image and head motion corrections. Head movement artifacts can lead to misinterpretation of the image data during analysis. Each 3D acquisition in a scan is collected in a small unit of 3D grid, which is referred to as a voxel. A single voxel represents a single image intensity value and ideally, voxels will always represent the same part of the brain in each acquisition, rather than vary from one 3D image to the next. To correct small head motion artifacts, AFNI' s motion correction tool employs a linear least squares algorithm that attempts to align each 3D image acquired to the first image acquired in the scan. In the preprocessing of fMRI BOLD data, we will also perform masking. A mask in image processing is analogous to filter in signal processing. The general purpose of filtering and applying masks is to remove unwanted signals, or rather unwanted pixels from an image. In our case, masking is necessary to remove the BOLD data outside of the brain region. Masking was also accomplished using AFNI.

AFNI offers a set of programs that can be used for batch processing using a shell script. We implemented an array of AFNI programs through a shell script to perform preprocessing of BOLD fMRI image data. AFNI further divides the NIfTI files into two separate file formats: 'BRIK' and 'HEAD'. The image binary i.e. the actual 3D volumes are stored in a '.BRIK' and the header information originally contained in the DICOM files is stored in a '.HEAD' file. AFNI uses these files as its inputs.

The following is a brief summary of the tasks performed during this step:

Preprocessing of BOLD FMRI Data	
Status during the reporting period	Completed
Actions	In the preprocessing of BOLD FMRI data we implemented a shell script to adjust the slice timing along with head motion corrections (Appendix D). The slice timing adjustment involves reconstruction of the fMRI image and head motion correction involves re-registrations of the voxels with respect to a base. Furthermore, the functional MR signals, or BOLD data outside the brain region was also masked out using a auto mask feature in AFNI.
Decisions	• Each 3D brain image is composed of multiple 2D slices and although the slices are acquired at essentially the same time, the duration of the scan separates the first scan from the last. So the 2D slices need to be aligned to the same point in time using interpolation. After this step the 2D image slices are reconstructed into an 3D image. • In an MRI imaging system, few MR signals generated at the very beginning of the scan are usually omitted. For this reason, the we excluded the first 5 TRs from our image data. • Generally, there lies some outliers in almost every statistical data. For head motion correction, we will use the TR with the least number of outliers in the BOLD EPI as the base.
Testing & Verification	The file generated at each step was visualized in AFNI. The visual comparisons and results are discussed in a bit more detail in section 3.

2.7.2 Preprocessing: Alignment of BOLD EPI to T1 Image

The second preprocessing step is to align the fMRI image, which was processed in the previous step, on top of the anatomical T1 weighted image. Structural MR image only provides information about brain anatomy. Nonetheless, the structural MRI provides enough information about the structure of the brain that complements functional MRI in a number of ways. Since, the brain function ultimately depends on the integrity of the brain structure, the underlying tissue integrity allows one to examine the functional signals. Essentially, structural MRI provides an anatomical reference for visualization of activation patterns and regions of interest to extract functional connectivity information. This step will allow us to map the BOLD signals in the EPI fMRI image to specific brain regions in the T1-weighted image.

Alignment of BOLD EPI to T1 Image	
Status during the reporting period	Completed
Actions	In this step, a shell script was run to align the BOLD EPI and the T1 image and to spatially normalize the aligned data to a standard template (Appendix E).
Decisions	• It was decided that we will use the standard TT_icmb452 brain atlas in talairach space as the template for normalizing our image data.
Testing & Verification	The file generated at each step was visualized in AFNI. The visual comparisons and results are discussed in a bit more detail in section 3.

2.8 Creating a Common GM Mask

Creating a Common GM Mask	
Status during the reporting period	Started
Actions	This is step a step in progress in our project. Here we will use AFNI to create a common grey matter mask by using the segmented data generated in the previous step.
Decisions	• The GM mask created initially created using the segmented image data was improper. Most of the pixels that were supposed to be there were missing. In the next attempt, we will generate another GM mask using the data that was aligned in the previous step.
Testing & Verification	The file generated after the first attempt were visualized in AFNI. After visualizations, we found most of the image pixels were missing form the GM mask.

3 Results and Discussions

The methods mentioned above are brought into implementation stepwise and its results are mentioned amd discussed below.

3.1 Data Selection and Conversion

The total fMRI data of 15 MDD and 15 HC of age group between 20 to 50 years with equal number of male and female were accurately obtained and both the chi square test and t-test resulted out to be true and those dataset were further converted to the NIfTI extension from DICOM format so at to establish the compatibility in AFNI environment.

(add picture of ms excel and NIfTI conversion what?)

3.2 Skull Striping

By running the command of 3dskullstrip, the scans of skull were removed from the structural images i.e, T1 images of all the subjects. The skull stripped structural images of all subjects were viewed in MRIcron so as to conform the error free command execution.

(skull stripped images)

3.4 nikin Image Segmentation Running the HC subjects for segmentation process, results the formation of rc1 images for gray matter, rc2 images for white matter and rc3 images for CSF that will be used for the common masking. (segmented images)

3.5 nikin Scripts Preprocessing

4 Conclusion and Further Work

As a closing note for the following progress report, the extracted dataset are statistically analysed so as to obtain the accuracy which would remove the differences that are created from manual analysis. All the MDD and HC subjects are also converted to NIfTI format to access in the AFNI formats from DICOM.Also, we successfully segmented the structural images of HC that resulted in isolation of gray matter, white matter and CSF which will further be used in common masking. Also, the images are skull stripped using AFNI commands to each subjects and are further used to preprocessed so that the images would be free from noises and any distortions by running bash scripts to each subjects.Thus these are the tasks that are performed so far to meet our objectives and successfully achieved our hypothetical results. Mentioning about our further works,in a preprocessed images,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.

References

[1] *Srpbs multidisorder mri dataset*, https://bicr-resource.atr.jp/srpbsopen/, 2021.

Appendix

A Data Selection

B Data Prep

```
#! /bin/bash

function appendDotnii () {
  for file in $(ls) ; do
    if [[ -f $file ]]
    then
      mv "${file}" "$(basename ${file}).nii"
    fi
  done
}

for category in $(ls -d */) ; do
  pushd $category
  for subject in $(ls -d */) ; do
    pushd $subject
    pushd rsfmri/
    appendDotnii
    popd
    popd
  done
  popd
done
```

C Skull Stripping

```
#! /bin/bash

WORKING_DIR=$HOME/Functional-Connectivity/Subjects/

# Create an array with the names of the directory in which the data
# for healthy controls and majorly depressed patient are stored.

CATEGORIES=(HC MDD)

# Change into the working directory if it exists

if [[ -d "${WORKING_DIR}" ]]
then
  pushd ${WORKING_DIR}
fi

if [ ! -d ''Skull_Stripped_Data'' ] ; then
    mkdir -p Skull_Stripped_Data/HC
    mkdir -p Skull_Stripped_Data/MDD
fi

for CATEGORY in "${CATEGORIES[@]}"
do
  pushd ${CATEGORY}

  for SUBJECT in $(ls)
  do
    pushd ${SUBJECT}/t1/

    SUBJECT_ID=$(echo "${SUBJECT}" | cut -d '-' -f 2)

    3dSkullStrip -input defaced_mprage.nii \
```

```
      -prefix defaced_mprage_${SUBJECT_id}.nii

    # Define your own path
    cp defaced_mprage_${SUBJECT_id}.nii \
      ../../../Skull_Stripped_Data/${CATEGORY}/

    popd
  done
  popd
done
```

D Preprocessing: BOLD FMRI Data

```
#! /bin/bash

###############################################################
# In this script you will preprocess BOLD data (4D fMRI data) #
# including slicing timing and head motion correction         #
###############################################################

WORKING_DIR=''$HOME/Functional-Connectivity/Processed_Data/''\
''BOLD_fMRI_Data''

# Create an array with the names of the directory in which the data
# for healthy controls and majorly depressed patient are stored.

CATEGORIES=(HC MDD)

# Move to the working directory

if [ -d "${WORKING_DIR}" ]
then
  pushd ${WORKING_DIR}
fi

for CATEGORY in "${CATEGORIES[@]}"
do
  pushd ${CATEGORY}

  for SUBJECT in $(ls *.nii)
  do
    SUBJECT_ID=$(basename -s .nii ${SUBJECT} | cut -d '_' -f 2)

    ###########################################################
    # Convert a dataset from NIfTI to .BRIK and .HEAD          #
    # -verbose because I want to be able to see what's going on #
    ###########################################################

    3dcalc -prefix ${SUBJECT_ID}-BOLD-EC-tmp                  \
      -a ${SUBJECT} -expr 'a' -verbose

    # Exclude the first 5 TRs
    # In the previous step, ${SUBJECT_ID}-BOLD-EC-tmp+orig.HEAD and
    # ${SUBJECT_ID}-BOLD-EC-tmp+orig.BRIK files were created.

    3dcalc -prefix ${SUBJECT_ID}-BOLD-EC1                     \
      -a ${SUBJECT_ID}-BOLD-EC-tmp+orig[5..$] -expr 'a'

    ## Despiking:

    3dDespike -prefix ${SUBJECT_ID}-BOLD-EC                   \
      ${SUBJECT_ID}-BOLD-EC1+orig

    ## Count the outliers in each TR

    3dToutcount -automask -range ${SUBJECT_ID}-BOLD-EC+orig   \
      > outliers-BOLD-EC.1D
```

```
    ## Find the TR with least outliers:
    ## This is a perl script.

    base=$(cat outliers-BOLD-EC.1D |                                \
      perl -0777an -F"\n" -e                                        \
      '$i=0;
       $small=999999;
       map {
         /\s*(\d+)/;
         if ($small > $1) {
           $small = $1;
           $ind=$i;
         };
       $i++;
       } @F;
       print $ind')

    # Using the TR with least outliers as base for head motion
    # correction and spatial normalization

    3dcalc -prefix ${SUBJECT_ID}-BOLD-EC-base                       \
      -a "${SUBJECT_ID}-BOLD-EC+orig[${base}]" -expr 'a'

    # Slice timing and head motion correction
    # head motion parameters are stored in
    # ${SUBJECT_ID}-BOLD-EC-motion.1D
    # This is the same with running 3dTshift first and then use
    # 3dvolreg itself. However, due to the lack of slice scan order,
    # we have to ignore this slice time step.

    3dvolreg -verbose                                               \
      -tshift 0                                                     \
      -base ${base}                                                 \
      -1Dfile ${subject}-BOLD-EC-motion.1D                          \
      -prefix ${SUBJECT_ID}-BOLD-EC-volreg                          \
      ${SUBJECT_ID}-BOLD-EC+orig

    rm -f ${SUBJECT_ID}-BOLD-EC+orig*

    ## Brain mask of the subject

    3dAutomask -prefix ${SUBJECT_ID}-BOLD-EC-mask                    \
      -dilate 1 ${SUBJECT_ID}-BOLD-EC-volreg+orig

    # Maskout the functional BOLD outside of the brain

    3dcalc -prefix ${SUBJECT_ID}-BOLD-EC-volreg-mask                 \
      -a ${SUBJECT_ID}-BOLD-EC-volreg+orig                           \
      -b ${SUBJECT_ID}-BOLD-EC-mask+orig -expr 'a*b'

    gzip *.BRIK
  done
  popd
done
```

E Preprocessing: Alignment of BOLD EPI to T1 Image

```
#! /bin/bash

# In this script we will spatially normalize the anatomical dataset
# into TT space, we will also use the same transformation to normalize
# the BOLD dataset into the MNI space.

WORKING_DIR="${HOME}/Functional-Connectivity/Processed_Data/"\
"Spatially_Normalized_Data"
```

```
# Create an array with the names of the directory in which the data
# for healthy controls and majorly depressed patient are stored.

CATEGORIES=(HC MDD)

# Change into the working directory if it exists

if [ -d "${WORKING_DIR}" ]
then
    pushd ${WORKING_DIR}
else
    echo "${WORKING_DIR}: doesn't exist"
    exit 111;
fi

# Use a nested loop to get into the directory for each subject of each
# category

for CATEGORY in "${CATEGORIES[@]}"
do
  pushd ${CATEGORY}

  for SUBJECT in $(ls)
  do

    # Grab the SUBJECT_ID
    # Subjects are stored in NORM-SUBJECT_ID
    # Split NORM-SUBJECT_ID at the delimiter and return the
    # specified field

    SUBJECT_ID=$(echo "${SUBJECT}" | cut --delimiter '-' --fields 2)

    pushd ${SUBJECT}

    ############################################################
    # Spatially normalize the anatomical dataset to MNI space #
    ############################################################

    # Uniformly distribute the white matter in the brain tissue.

    3dUnifize -prefix BOLD-${SUBJECT_ID}-EC-T1                    \
        -input ANAT-${SUBJECT_ID}.nii

    # Remove the skull and extract the brain tissue from T1-weighted
    # MR image.

    3dSkullStrip -prefix ${SUBJECT_ID}-T1-NoSkull                 \
        -input BOLD-${SUBJECT_ID}-EC-T1+tlrc.

    # Shift the center of DSET to the center of BASE. '_shft' will be
    # appended at the end of DSET. Use the center of mass of the
    # volume as the center (By default, center is the center of
    # volume's grid).
    @Align_Centers -cm                                            \
        -base ${SUBJECT_ID}-BOLD-EC-base+orig.                    \
        -dset ${SUBJECT_ID}-T1-NoSkull+tlrc.

    # Linearly align the anatomical dataset with the EPI dataset. The
    # EPI dataset cab be the functional-MR image. The -epi_base option
    # specifies the starting sub-brick for the alignment.

    align_epi_anat.py -anat ${SUBJECT_ID}-T1-NoSkull_shft+tlrc.   \
        -epi ${SUBJECT_ID}-BOLD-EC-base+orig.                     \
        -epi_base 0                                               \
        -suffix _alBOLDEC                                         \
        -anat_has_skull no                                        \
        -epi_strip 3dAutomask                                     \
        -volreg off                                               \
        -tshift off                                               \
        -resample off
```

```
# Create a symbolic link for the atlas you want to use.  Atlases
# are downloaded during AFNI installation. To find the location of
# the atlases run `afni_system_check.py -check_all | grep atlas`.
# If the atlases were not downloaded; download them from
# "https://bit.ly/3BpKN2K". Once the tarball has been extracted
# and placed into whichever location the afni binaries are stored
# in, they are immediately available for use.

# We will use TT_icbm452+tlrc atals

ln -sf /opt/afni/TT_icbm452+tlrc.* ./

# Shift (roughly) the center of the aligned anatomical dataset
# with the standard template. The transformation information will
# be stored in the base image of BOLD data as well. Again,
# "_shft" will be appended to the DSET and CHILD.

@Align_Centers -base ./TT_icbm452+tlrc.                        \
    -dset  ${SUBJECT_ID}-T1-NoSkull_shft_alBOLDEC+tlrc.        \
    -child ${SUBJECT_ID}-BOLD-EC-base+orig.

# Align SOURCE to BASE and save the transformation matrix for each
# sub-brick into a 1D file. The cost function that defines the
# matching between SOURCE and BASE is the lpa (Local Pearson
# Correlation Abs). Also comput a weight function using 3dAutomask
# alogrithm plus some blurring of the base image.

3dAllineate -prefix ${SUBJECT_ID}-T1_to_T1_Allineate           \
    -base ./TT_icbm452+tlrc.                                   \
    -source ${SUBJECT_ID}-T1-NoSkull_shft_alBOLDEC_shft+tlrc.  \
    -1Dmatrix_save T1_to_T1_Allineate.aff12.1D                 \
    -source_automask                                           \
    -cost lpa                                                  \
    -autoweight                                                \
    -cmass

# Compute a non-linearly warped version of SOURCE dataset to match
# the BASE dataset. Gaussian blur the SOURCE and BASE before doing
# the alignmnet. By default, the blur values is 2.345 (for no good
# reason). Set the blur values to 0 if you do not want to blur the
# inputs.

3dQwarp -prefix T1-NoSkull_shft_alBOLDEC_shft                  \
    -blur 0 0                                                  \
    -base ./TT_icbm452+tlrc.                                   \
    -source ${SUBJECT_ID}-T1_to_T1_Allineate+tlrc

# So, the 3dcopy command seems to overwrite one of the files
# created above. The old file needs to be deleted for 3dcopy to
# execute. ${SUBJECT_ID}-T1-NoSkull_shft_alBOLDEC_shft+tlrc.* will
# be recreated by 3dcopy.

rm --force ${SUBJECT_ID}-T1-NoSkull_shft_alBOLDEC_shft+tlrc.*

# Copy one dataset to the other. 3dCopy foo bar copies foo+orig.
# to bar+orig. and foo+tlrc. to bar+tlrc. This program copies
# entire datasets and not jsut sub-bricks.

3dcopy T1-NoSkull_shft_alBOLDEC_shft                           \
    ${SUBJECT_ID}-T1-NoSkull_shft_alBOLDEC_shft

######################################################
# Spatially normalize the BOLD dataset to MNI space #
######################################################

# Create a copy of the original volume registartion mask.

3dcopy ${SUBJECT_ID}-BOLD-EC-volreg-mask+orig.                 \
    ${SUBJECT_ID}-BOLD-EC-volreg-mask_temp
```

```
    # Shift (roughly) the center of the 4D BOLD fMRI data to the space
    # using the same parameters as the base image. The base image has
    # similar alignmnet as the anatomical image. (As explained
    # earlier).

    @Align_Centers -cm                                              \
        -base ${SUBJECT_ID}-BOLD-EC-base_shft+orig                  \
        -dset ${SUBJECT_ID}-BOLD-EC-volreg-mask_temp+orig.

    ## Apply a nonlinear transformation and resample to 3x3x3 mm

    3dNwarpApply -prefix ${SUBJECT_ID}-BOLD-EC-volreg-Nwarp         \
        -source ${SUBJECT_ID}-BOLD-EC-volreg-mask_temp_shft+orig. \
        -nwarp 'T1-NoSkull_shft_alBOLDEC_shft_WARP+tlrc. '\
        'T1_to_T1_Allineate.aff12.1D'

    3dcopy ${SUBJECT_ID}-BOLD-EC-volreg-Nwarp+orig                  \
        ${SUBJECT_ID}-BOLD-EC-volreg-Nwarp_Backup

    3drefit -view tlrc ${SUBJECT_ID}-BOLD-EC-volreg-Nwarp+orig

    ## get tissuse-based signal before smooth

    3dDeconvolve -float -polort A                                   \
        -errts ${SUBJECT_ID}-BeforeSmooth-lp                        \
        -bucket BeforeSmooth-bucket                                 \
        -input ${SUBJECT_ID}-BOLD-EC-volreg-Nwarp+tlrc.

    3dBandpass -band 0.01 0.08                                      \
        -prefix ${SUBJECT_ID}-BeforeSmooth-lp-bp                    \
        -input ${SUBJECT_ID}-BeforeSmooth-lp+tlrc

    # Spatial smoothing with 6 mm FWHM. The value for FWHM is
    # adjustable

    3dmerge -doall                                                  \
        -1blur_fwhm 6                                               \
        -prefix ${SUBJECT_ID}-BOLD-EC-volreg-Nwarp-blur6            \
        ${SUBJECT_ID}-BOLD-EC-volreg-Nwarp+tlrc

    # Linear detrending

    3dDeconvolve -float -polort A                                   \
        -bucket bucket                                              \
        -errts ${SUBJECT_ID}-BOLD-EC-volreg-Nwarp-blur6-lp          \
        -input ${SUBJECT_ID}-BOLD-EC-volreg-Nwarp-blur6+tlrc.

    # Temporal filtering band pass 0.01-0.08 Hz

    3dBandpass -band 0.01 0.08                                      \
        -prefix ${SUBJECT_ID}-BOLD-EC-volreg-Nwarp-blur6-lp-bp      \
        -input ${SUBJECT_ID}-BOLD-EC-volreg-Nwarp-blur6-lp+tlrc

    3dresample -master ./TT_icbm452+tlrc.                          \
      -input ${SUBJECT_ID}-BOLD-EC-volreg-Nwarp-blur6-lp-bp+tlrc. \
      -prefix ${SUBJECT_ID}-BOLD-EC-volreg-Nwarp-blur6-lp-bp-resampled

    gzip *.BRIK
    popd
  done
  popd
done
popd
```

F Gantt Chart (Unchanged)

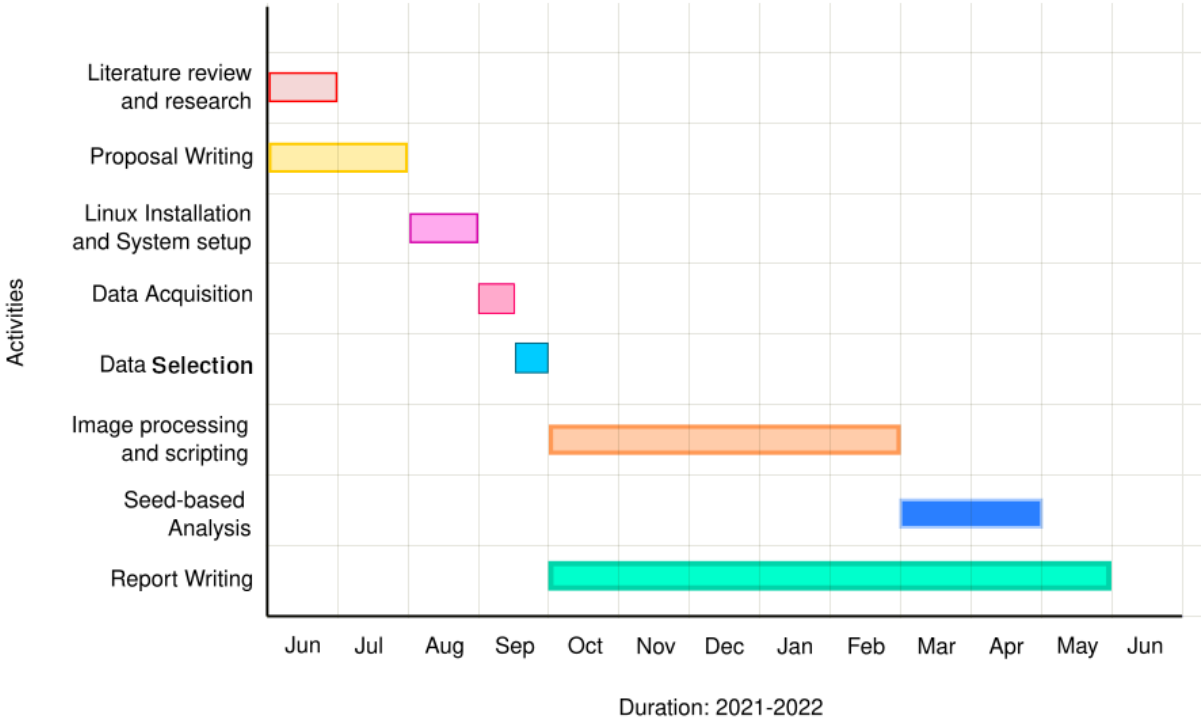


Figure 2: Proposed Workflow (Unchanged)