# Purbanchal University

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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.

The following progress report 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.

- Authors

# Abstract

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## **Abbreviations**

**AFNI** Analysis of Functional Neuroimages

**BDI** Beck Depression Inventory

**BOLD** Blood Oxygen Level Dependent

**DSM** Diagnostic and Statistical Manual of Mental Disorders

fMRI Functional Magnetic Resonance Imaging

**HC** Healthy Controls

**MDD** Major Depressive Disorder

MR Magnetic Resonance

**ROI** Region of Interest

rs Resting State

rsFC Resting-state Functional Connectivity

**SCA** Seed-based Correlation Analysis

sMRI Structural Magnetic Resonance Imaging

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## 1 Introduction

## 1.1 Background

Major Depressive Disorder being one of the major mental health problems, our project aims to ponder upon the performance of the seed based functional connectivity analysis on MDD patients deploying computational tools with implementation of the image preprocessing algorithms. 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.

To meet our objective, we employed various computational tools like MS Excel, MRIcron, Octave, SPM(Statistical Parametric Mapping), AFNI (Analysis of Functional Neuroimages) in a LINUX Operating Environment. All these tools are open-source softwares that added feasibility to the progress of our objectives. Our main focus is on the functional connectivity analysis of the hippocampal network of the MDD patient compared to that of healthy control. Publicly available dataset was used in this project. The MR images of MDD patients as well as that of healthy individuals for this project are retrieved from 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). Statistical analysis is performed on the selected dataset to lead our project more to accuracy as manual analysis may create more chaos. After this, those analysed data are converted to nifti extension from DICOM followed by the conversion from 3D to 4D MRI Images. Meanwhile, the structural brain MR-images are subjected to segmentation process through SPM. Further, the functional brain MR images are preprocessed. These tasks have been accomplished while our end goal is yet to be achieved which is to analyze the preprocessed images on the basis of their functional connectivity of both MDD and HC patients, comparing them and presenting the analysis.

#### 1.2 Rationale

The diagnosis procedures that are the gold standard for the diagnosis of psychiatric disorders are wholly based on behavioral observations and patient reported symptoms. This doesn't completely diagnose the mental disorder that would lead to the preferable treatment for the same that would result proper cure. So, neuroimaging basis can play significant role to lead the diagnosis of mental disorders which is why the functional connectivity analysis of the resting state fMRI of metal disorder patients can boom the medical diagnosis for the same leading to proper cure and treatment. As hippocampus, the region responsible for emotion and memory processing, is one of the major region of the brain that is said to be affected on the person suffering from mental disorders, this project represents the novelty for the major changes in the mental disorder diagnosis. 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 on various part of the brain. Thus, this project can lay the foundations for further research and development.

# 2 Methodology

Progress of this project includes Statistical t-test analysis of the separated data of both MDD and HC patients, deploying various computational tools for conversions, segmentation and preprocessing of the images till the date. First of all, literature review of papers and articles was done which helped us boost our theoretical knowledge and develop our own strategy to meet our project's objective.

#### 2.1 fMRI Data Selection and Statistical Analysis

In this project, Data selection includes retrieving disparate types of data from DecNef Project Brain Data Repository. We retrieved 30 MRI images of public data set out of which 15 are MDD and 15 are HC. We, first, preferred to take the dataset with respect to the age and sex lying between 20 to 30 years age group but since the required dataset was not enough as per our decided number of patients, we extracted the patients of age group lying between 30 to 50 years. Also for gender, we decided to take equal number of male and female data, for that we selected equal number of male and female manually and to verify that, we performed Chisquared test. To perform that test, we created a pivot table in MS Excel and then, we inserted the number of male and female in the pivot table. Then, applying the formula of chi-squared test in the table, we verified that the number of male and female data are equal. Now, for the age group, we performed t-test, so, we acquired the age of both MDD patients and HC in two rows and in MS Excel, we applied t-test (two tailed distribution) to check the validation of age group.

#### 2.1.1 Chi-squared test

A chi-squared test is a statistical hypothesis test that is valid to perform when the test statistic is chi-squared distributed under the null hypothesis. Pearson's chi-squared test is used to determine whether there is a statistically significant difference between the expected frequencies and the observed frequencies in one or more categories of a contingency table. The purpose of the test is to evaluate how likely the observed frequencies would be assuming the null hypothesis is presence of manually selected number of Male and Female which resulted out to be true.

#### 2.1.2 t-test

A t-test is a type of inferential statistic used to determine if there is a significant difference between the means of two groups, which may be related in certain features. A t-test looks at the t-statistic, the t-distribution values, and the degrees of freedom to determine the statistical significance. [https://www.investopedia.com/terms/t/t-test.as p] So, we performed this test for the verification of the presence of desired i.e, 20 to 30 years of age group of both MDD ans HC patients. Thus, from the large SRPBS public dataset, we extracted 15 MDD and 15 HC patients whose age group lie between 30 to 50 age group including equal number of male and female having BDI Index greater than 3 that indicated the accuracy to our project's analysis.

#### 2.2 Data Conversion

Next step includes data conversion of extracted one i.e, the obtained public dataset is in DICOM format so, we basically converted those extracted data to NIFTI format for further processing compatibility. For this, in linux environment, we installed ,MRIcron (magnetic resonance image conversion, viewing and analysis) was installed using command 'sudo apt-get install' MRIcron so that we can load multiple layers of images, create volume renderings and draw region of interest. It also provides 'dcm2nii' tool that can convert DICOM format images to NIFTI format.DICOM(Digital Imaging and Communications in Medicine) is a standard, internationally accepted format used to view, store, retrieve and share medical images. NIfTI is the format that attempts to keep spatial orientation information. Many of the popular tools used for scientific image processing, analysis and visualization require images to be stored in the NIfTI file format, whereas scanners used to acquire these images usually export data in the DICOM format. DICOM standard is complicated and different scanner manufacturers extend the DICOM standard in a variety of ways, often resulting in duplication of information and incompatibilities between software only designed to work with one particular subset of DICOM. Therefore, while one conversion tool may work for many images, it may fail for others. So, the DICOM format images are converted to NIfTI format. During the installation it didn't meet the dependencies so the dependencies were corrected using command 'sudo apt -fix-broken install'. 'which dcm2niigui' command was used to check if the dcm2nii tool is present or not, '/usr/bin/dcm2niigui' if appeared then it indicates dcm2nii tool is installed. dcm2nii can be used in two ways either by dragging and dropping files onto the program or by launching dcm2nii from command line and specifying the options to use. We used the drag and drop method: firstly all the DICOM format images were placed into a folder followed by dragging and dropping of images onto dcm2nii one by one. The File/ModifyNIfTI command can be used to change existing NIfTI images which can guide us through selecting the images and choose how we'd like to modify the images(remove volumes, changing sub format, reorienting, or changing the order of the 3rd and fourth dimension). The Neuroimaging Informatics Technology Initiative (NIfTI) provides coordinated and targeted service, training, and research to speed the development and enhance the utility of informatics tools related to neuroimaging. NIfTI focuses on tools used in fMRI because fMRI is rapidly growing, and improvement in informatics tools will provide broad benefits for neuroscience and greater cost/benefit ratio. NIfTI is more useful and useable neuroimaging informatics tools that provides environment to facilitate convergence on common solutions to widespread problems and maximizes scientific opportunities from neuroimaging research. This format has been widely adopted in neuroimaging research, allowing scientists to mix and match image processing and analysis tools developed by different teams. For slice orientation other than axial, the image could be re-organized as if it were axial slice. This won't cause any problem in terms of the NIfTI format, and may avoid confusion in some analysis and visualization tools. ( write about the script for nifti conversion Bloody no)

## 2.3 Skull stripping of rsfmri Images

Skull stripping is the process of isolating brain tissue from non-brain tissue from an MRI image of a brain. [https://www.analyticsvidhya.com/blog/2021/06/introduction-to-skull-stripping-image-segmentation-on-3d-mri-images/] Skull stripping may improve the robustness of the registration process, since high resolution structural images contain considerable amounts of non-brain tissue such as eyeballs, bone, skin, and other tissues. This step shows general improvement in the quality of the normalization and template congruence for skull-stripped images in every tissue category (whole brain, gray matter, white matter). Skull Stripping improves registration and normalization. Skull stripping consists of following three steps: 1) Volume is preprocessed to exclude gross spatial image non-uniformity artifacts the brain is repositioned in reasonable manner. 2) A spherical surface is iteratively expanded until the brain is enveloped. 3) Various masks and surfaces that models the brain and portions of the skull are created.

3D skull-stripping is done on T1 image(structural images of brain) using the command: Command: 3dSkullStrip -input filename -prefix

We run this command in LINUX terminal by selecting the T1 images of specific folders of MDD and HC patients, we isolated the brain tissue from non-brain tissue of all T1 images of MDD and HC patients. So as to view the isolated brain tissue of fMRI data of each patient's brain, we viewed the skull stripped brain images on the MRIcron by properly selecting the T1 images of each MDD ad HC subjects.

## 2.4 Image Segmentation

Segmentation is the most important part in image processing. Fencing off an entire image into several parts which is something more meaningful and easier for further process. These several parts that are rejoined will cover the entire image. Segmentation may also depend on various features that are contained in the image. It may be either color or texture. Before de-noising an image, it is segmented to recover the original image. The main motto of segmentation is to reduce the information for easy analysis. Segmentation is also useful in Image Analysis and Image Compression.cite1

In our project, segmentation is done to identify grey matter, white matter and CSF from the fMRI images of the brain. So, we proceed the segmentation process using SPM(Statistical Parametric mapping). Segmentation in SPM can work with images collected using a variety of sequences, but the accuracy of the resulting segmentation will depend on the particular properties of the images.

SPM (Statistical Parametric Mapping) is an fMRI analysis software package that is run in Matlab. So, first installed MATLAB which would require the MATLAB licensing that would ultimately add cost to our project. Thus, We preferred to install Octave instead of MATLAB since Octave being open source software.

#### **2.4.1** Octave

Octave is a high-level interpreted language, primarily intended for numerical computations. It provides capabilities for the numerical solution of linear and nonlinear problems, and for performing other numerical experiments. It also provides extensive graphics capabilities for data visualization and manipulation. Octave is also known as GNU Octave. It is mainly used in solving the linear and non linear problems numerically, and for performing numerical experiments it is mostly compatible with MATLAB. It mainly consists of function calls or script. Its syntax is mainly matrix-based and provides various functions for matrix operations. Octave does support various data structures and object-oriented programming. It has great features and compatible with other languages like syntax and functional compatibility for MATLAB. It shares other features like built-in support for complex numbers, powerful built-in math functions, and extensive function libraries and in terms of user-defined functions as well. Octave is normally used through its interactive command line interface, but it can also be used to write non-interactive programs. The Octave language is quite similar to Matlab so that most programs are easily portable.

#### 2.4.2 SPM12

SPM(Statistical Parametric Mapping) refers to the construction and assessment of spatially extended statistical processes used to test hypotheses about functional imaging data.citeOSPM runs in the octave package. SPM requires the image data in a suitable format. Here, data used is in nifti format. There are two main forms of nifti – here used form is ".nii"files , which combines all the information into one file. Nifti images can be single 3D volumes, but 4D or even 5D images are possible. VBM Pre-processing in SPM12 include: Use of Segment for characterising intensity distributions of tissue classes, and writing out "imported" images that DARTEL can use. Run DARTEL to estimate all the deformations.

#### 2.4.3 Image Segmentation Process

Segmentation is done to identify grey and white matter. Grey matter contains high densities of unmyelinated (lacking a myelin sheath) neurons, white matter contains high densities of myelinated neurons and CSF is contained in large amount in ventricles. Segmentation in SPM can work with images collected using a variety of sequences, but the accuracy of the resulting segmentation will depend on the particular properties of the images. Although multiple scans of each subject were available, the dataset to be used only includes the T1-weighted scans. For greater accuracy of registration, the tissues of our anatomical image need to be accurately mapped to the tissues of template. Segmentation can be found within SPM-Spatial-Segment in the batching system. The output of segmentation will be used for achieving more accurate intersubject alignment using DARTEL. In this project, data used were a selection of T1-weighted scans of healthy control(HC).

#### 2.4.4 Image Segmentation Algorithms

There are various techniques used in image segmentation.following are some methods:

- 1) Region based segmentation Region based segmentation is of two types:
- a) Threshold Segmentation: In this segmentation technique, image gray scale information processing is directly divided based on the gray value of various targets. Segmentation effect

can be obtained if the target and background have high contrast. following are the types of Threshold segmentation:

- i) local threshold method: In this method multiple segmentation thresholds are selected and images are divided into multiple target regions and backgrounds using multiple thresholds.
- ii) Global threshold method: In this method image is divided into two regions of target and background using a single threshold.
- b) Regional growth segmentation: Firstly a seed pixel is selected and eventually similar pixels are merged around seed pixel into the region, where seed pixel is located.

2)Edge detection segmentation:

A gray edge between two adjacent regions with different gray values is always present in the image and case in which the gray value is not continuous. Those discontinuities are detected using derivative operations(derivatives are calculated using differential operators). Generally, Parallel edge detection is used as a technique of image preprocessing, which is done by means of a spatial domain differential operator so as to perform image segmentation by convolution of its template and image.

3)Segmentation based on clustering:

In this technique, the pixels in image space are segmented with corresponding feature space, which is then segmented and mapped back to original image space so as to generate segmentation outcome. k-means is the most common clustering algorithm used. It basically gathers samples into different clusters based on the distance. For the achievement of compact and independent clusters as clustering targets the two points need to closer.

As we are mainly concerned with the grey matter, white matter and CSF of HC fMRI Images, we chose the tissues associated to grey matter, white matter and CSF only along with the respective native tissues being selected as "Native+DARTEL". After everything had been set up, the image segmentation starts. As a result, there should be a bunch of new images files generated. Files containing "c1" in their name are what the algorithm identifies as grey matter. If they have a "c2" then, they are supposed to be white matter. The "c3" images, are CSF.

#### 2.4.5 DARTEL

DARTEL (Diffeomorphic Anatomical Registration Through Exponentiated Lie Algebra) The idea behind DARTEL is to increase the accuracy of inter-subject alignment by modeling the shape of each brain using millions of parameters. DARTEL works by aligning grey matter among the images, while simultaneously aligning white matter. This uses the imported "rc1" and "rc2" images, and generates "u\_rc1" files, as well as a series of template images. Main idea of DARTEL is to register images by computing a flow field which can then be exponentiate to generate both forward and backward deformations .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 a 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.

## 2.5 Image Preprocessing

fMRI image preprocessing 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 application we are working for. An fMRI volume contains not only the signal that we are interested in, changes in oxygenated blood but also fluctuations that we are not interested in, such as head motion, random drifts, breathing, and heartbeats. These other fluctuations are noise, which are to be separated from the images or its region that we are interested in.

fMRI images are of lower resolution because they are collected at a very fast rate. So, they are needed to be

1) reconstructed 2) renamed, 3) made into 3D bricks 4) reregistered (to correct for head movement), 5) deconstructed 6) formatted into another type of image file, and 7) normalize

#### 2.5.1 AFNI

Analysis of Functional Neuro Images is an open-source environment for processing and displaying functional MRI data which is a technique for mapping human brain activity. AFNI is a set of programs that can be used interactively or flexibly assembled for batch processing using shell script. It is developed for the analysis and display of multiple MRI modalities such as anatomical, functional MRI (FMRI) and diffusion weighted data. It is freely available for research purposes. AFNI allows researchers to overlay a functional image to the anatomical, providing tools for aligning the two into the same space.

The AFNI performs the following pre-processing steps and finish along with a basic regression analysis:

1.Slice timing: Each 3D brain image is composed of multiple 2D images or slices. Although acquired at approximately the same time, up to several seconds could separate the first slice acquired from the last. Through interpolation, the slices are aligned to the same time point. Generally, any introduced noise from interpolation errors is thought to be outweighed by improvements in signal.

2.Motion correction: Head movements can create sources of error in the analysis. Each 3D acquisition in a scan is collected on a 3D grid, with each small cube of grid space which is also called voxel. Representing a single image intensity value. 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 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.

3.Smoothing: To reduce random noise in the image, a smoothing is applied. While smoothing can increase the signal-to-noise ratio of the image, it reduces image resolution.

4.Mask: Removes any non-brain areas, such as skull, from the fMRI image. 5.Scale: Scale each voxel so that changes in intensity represent percentage of signal change over the course of the scan. The default sets the mean of each voxel equal to 100.

To initialize the preprocessing steps sequentially, we run various scripts as indicated

- 1) Prep-2-BOLD-EC-preproc.sh 2) Prep-3-BOLD-EC-align-to-anat-MNI1.sh 3) Prep-7-reCovariates All these scripts contains various commands that are used in image preprocessing in AFNI.
- 1) Prep-2-BOLD-EC-preproc.sh

3dcalc - What it does- This program does voxel-by-voxel arithmetic on 3D datasets. Why we used it- To convert DICOM files to AFNI format and then exclude the first 4 TRs.

3dDespike - What it does- It removes 'spikes' from the 3D+time input dataset and writes a new dataset with the spike values replaced by something more pleasing to the eye.

3dToutcount -automask: It calculates number of 'outliers' a 3D+time dataset, at each time point. So it is used to count the outliers at each TRs. Using base command, TR with less is detected and use that TR with least outliers as base for head motion correction and spatial normalization.

3dvolreg- The AFNI command to perform motion correction and estimate spatial deviations between the reference functional image and other functional images using each of the movement parameters like base, tshift

3dAutomask- It is used for the brain masking of the obtained subject.

3dcalc- It helps to maskout the functional BOLD outside of the brain.

2) Prep-3-BOLD-EC-align-to-anatomical-MNI1.sh This scripts includes following command that are run on the

3dUnifize - What it does- Bascially white matter in T1-weighted images is made reasonably uniform in intensity,through the brain regions. Why we used it-we use the 3dUnifize program to (approximately) spatially uniformize and normalize intensities throughout the brain region, which helps in the matching process, especially when using datasets from different scanners.

@Align\_Centers- We've used @Align\_Centers twice in the script. 1st)Moves the center of DSET to the center of BASE.BASE: Base volume, typically a template. DSET is typically an anatomical dset to be aligned to BASE. Options used: -cm: Center is the center of mass of the volume. Why we used it- to roughly move the center of the base image of BOLD data with the anatomical data of each subject. 2nd)roughly moves the center of the aligned anatomical data with standard anatomical template in the TT\_icmb space. Options used: -child: A bunch of datasets, originally in register with DSET, that should be shifted in the same way.

align\_epi\_anat.py- This Python script computes the alignment between two datasets, typically an EPI and an anatomical structural dataset, and applies the resulting transformation to one or the other to bring them into alignment. Options used: -volreg off: to not perform volume registration on EPI dataset before alignment -tshift off: to not perform time shifting of EPI dataset before alignment -anat2epi: align anatomical dataset to EPI dataset (default) master\_anat: -master grid resolution for anatomical to epi output -epi\_base: Base subbrick to use for alignment. Choose sub-brick number or statistic type(for example, 0,5,mean) -anatomical\_has\_skull no: anatomical is assumed to have no skull epi\_strip:method to mask brain in EPI data. Why we used it- to linearly align the anatomical data to BOLD data.

In - This makes link between files.

3dAllineate- It is a program to align one dataset (the 'source') to a 'base' dataset, using an affine (matrix) transformation of space. Options used: -cmass = Use the center-of-mass calculation to determin an initial shift [This option is OFF by default] -autoweight = Compute a weight function using the 3dAutomask algorithm plus some blurring of the base image. lpait allows autoweight -source\_mask sss = Mask the source (input) dataset, using 'sss

3dQwarp-This program computes a nonlinearly warped version of source\_dataset to match base\_dataset. Why we used it? - To produce a dataset warped to match the TTicbm template. Since the I152 template is already somewhat blurry, the amount of blurring applied to it is set to zero, and also the source dataset will be Gaussian blurred with a FWHM of 0 mm. After using 3dQwarp he source dataset is warped to match the base and gets prefix as output.

3dCopy - This program will copy all datasets using the old\_prefix to use the new\_prefix.

3dNwarpApply - Program to apply a nonlinear 3D warp saved from 3dQwarp to a 3D dataset, to produce a warped version of the source dataset. Options used: -master mmm = 'mmm is the name of the master dataset, which defines the output grid. -nwarp option has two filenames inside single quotes,this feature tells that program to compose (catenate) those 2 spatial transformations before applying the resulting warp. Why we used it? -to apply the nonlinear transformation and resample to 3x3x3 mm

3drefit -This program changes some of the information inside a 3D dataset's header. Options used: -view code- Changes the 'view' to be 'code', where the string 'code' is one of 'orig', 'acpc', or 'tlrc'. Why we used it?- to convert orig view to tlrc view.

3dDeconvolve - Program to calculate the deconvolution of a measurement 3D+time dataset with a specified input stimulus time series. Options used: [-polort pnum]- -polort option allows the use of 'A' to set the polynomial order automatically. The purpose of '-polort' is to build

the columns of the regression matrix. Why we used it? 1-to get tissuse-based signal before smooth. 2-linear detrending

3dBandpass-This program is similar to 3dFourier. This is a program to lowpass and/or highpass each voxel time series in a dataset. Options used: -band fbot ftop = Alternative way to specify passband frequencies. fbot = lowest frequency in the passband, in Hz ftop = highest frequency in the passband (must be > fbot) -retrend -any mean and linear trend are removed before filtering.

3dmerge - This program has 2 different functions: (1) To edit 3D datasets in various ways (threshold, blur, cluster, ...); (2) To merge multiple datasets in various ways (average, max, ...). Either or both of these can be applied. Options used: -doall = Apply editing and merging options to all sub-bricks uniformly in a dataset. Why we used it? -For spatial smoothing. Specifically:to apply a 6mm FWHM(Full Width at Half maximum) Gaussian blur

3) Prep-7-reCovariates (left)

#### 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 grey 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

In a nutshell, 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 grey 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

# **Appendix**

# A Hello