CHAPTER 3

SUBJECTS AND METHODOLOGY

3.1 INTRODUCTION

The main aim of this research work is to analyze EEG of SCH at various recording conditions such as rest, HV, PHV and photic conditions along with EEG during mental activity and identify the variations with reference to EEG of normal subjects. This chapter explains about the method of EEG recording and the nature of subjects.

3.2 SUBJECTS

Any physiological recording for research purpose requires study procedure and the details of inclusion and exclusion criteria for subjects to be approved by Ethics committee. The study design methodology is approved by the Institutional Ethics committee of Madras Medical College, Chennai, India and the certificate of approval is attached in Appendix 1. The work is being carried out at Institute of Mental Health, Chennai, India. The subjects who are diagnosed as SCH by doctor alone are included in this study. The subjects who have other psychotic symptoms and having neurologic complaints are the exclusion criteria for this study. The protocol involves recording conventional EEG and during the mental activity. The conventional method of EEG recording follows the conditions namely, eyes closed, HV, PHV and Photic Stimulation (PS) at various driving frequencies. In continuation with this, EEG is to be recorded during the mental task given. Therefore, the subjects
who can stay stable and can understand the cues while recording are suggested by the doctors for this study.

Initially, all SCH subjects are assessed by psychiatrist based on either the self-reported experience given by them or by their first degree relatives. It is necessary that the subjects should go for the MSE, but their score is not considered as a factor in this research. The MSE is carried out by the psychologist who does the necessary observations and investigations as discussed in section 1.1.3. As per the MSE examination report given by psychologist and based on the observations, the subjects who fulfill DSM-IV criteria are declared as SCH by the psychiatrist. However, this study considers the SCH subjects irrespective of the subtypes of SCH. The SCH subjects whoever involved in this research are under antipsychotic medications. The method of recording EEG is explained in the following section. Totally 104 subjects are considered for this study. The head band is placed over the scalp in order to fix the electrodes in the respective positions. Then after applying electrode gel, the electrodes are fixed.

Some subjects are unable to cooperate during entire recording. Therefore, finally EEG from 81 subjects are recorded. Out of 81 subjects, 52 are SCH and the remaining 29 are normal subjects. In SCH subjects group, 23 female with average age of 46 and 29 male subjects with average age of 41 are considered. Thus the total average age of SCH subjects group is 44. 12 female and 17 male normal subjects group are considered in this study. Their average age is 43 and 41 respectively. Thus, the total average age of normal subject group is 42. The other factors such as weight and height are not taken into consideration in this study, because symptoms of SCH do not have relationship with height and weight of a subject. The study design methodology is explained to the subjects and informed consent is obtained. Sample consent form is shown in Appendix 2.
3.3 EEG RECORDING PROTOCOL

The EEG signal has to be acquired from the subjects and for this conventional standard EEG machine is used. The 24 channel Brain Clarity – Brain Tech +40 equipment is used to record EEG at 19 electrode locations namely Fp2, F4, C4, P4, F8, T4, T6, O2, Fp1, F3, C3, P3, F7, T3, T5, O1, Fz, Pz, and Cz of International standard 10-20 system. In addition to these positions, the electrodes are also fixed at A1, A2 which are reference points. From the literature (Nash Boutros et al. 2008), it is observed that most of the EEG studies used monopolar montage as this type of montage provides well localized potential and hence in this research work also EEG is recorded using monopolar montage with linked ear (A1 & A2) as a reference. The electrodes are placed over the head as shown in Figure 3.1. Figure 3.1, shows international standard 10-20 electrode systems for EEG recording. The numbers 10 and 20 represents the 10% and 20% distances between the adjacent electrodes where 100 % is the reference total distance from nasion (root of the nose) to inion (ossification center). Similarly, electrodes are placed from left auricular point to right auricular point. Each position is identified by letters F, C, P, T and O which means frontal, central, parietal, temporal and occipital lobes respectively and even and odd numbers present in the suffix represents electrode positions on the right hemisphere and left hemisphere respectively. Similarly, letter z and p in the suffix represents the electrodes placed on the midline and prefrontal positions respectively. The ear lobe electrodes A1 and A2 are electrodes placed on the auricular points. First a line must be drawn from nasion to inion and similarly between left auricular and right auricular point. The intersection of these two lines is marked as Cz. From nasion to inion 10%, 20%, 20%, 20%, 20% and 10% of the total distances, electrode positions Fpz, Oz, Fz, Pz, and Cz are marked respectively. But Fpz and Oz are not shown in Figure 3.1. Similarly C4, T4, T6, C3, T3, and Cz positions are marked between the auricular points. The outer circle
electrodes are placed in both left (F_{P1}, F_{T3}, T_{T5}, O_{1}) and right hemisphere (F_{P2}, F_{T4}, T_{T6}, O_{2}) between F_{PZ} and O_{Z} marked accordingly 10%, 20%, 20%, 20%, 20% and 10% of the total distance. The electrodes C_{3}, F_{3} and P_{3} are marked equally 20% distance from F_{P1} to O_{1}. Similarly, the other electrodes are fixed in the right hemisphere.

Figure 3.1 Placements of Electrodes

Out of 19 scalp electrode positions mentioned above, for the linked ear reference, the potential from the electrodes positions of F_{Z}, P_{Z} and C_{Z} are not utilized for the monopolar montage because these electrodes are located at equal distance from both A_{1} and A_{2} reference electrodes and hence treated as common point for both left and right hemisphere. Hence only the remaining 16 electrode positions are considered for this montage. The sampling frequency of the EEG equipment is kept at 256 Hz. EEG signals are filtered by a band pass filter with upper and lower cut-off frequencies of 0.1 Hz and 70 Hz respectively and notch filter is set on (50 Hz) to remove power line interference. The sensitivity of the equipment is set to 7.5 µv/mm and the recording speed is 30 mm/s. EEG recording room is acoustically isolated which is far from large motors, elevators, and large A/C equipment to avoid external disturbance while recording EEG.
3.4 EEG CONVENTIONAL RECORDING

EEG is recorded during the condition namely rest, HV, PHV, PS condition and the protocol is explained below. The recording begins with the explanation of the aim of the research and study procedure being informed to the subjects. Initially, the subjects are asked to sit in a chair in a relaxed position with closed eyes. EEG recording started with closed eyes which took 1 minute to ensure the baseline record and within this period, the subjects are asked to just open and close the eyes.

The HV protocol for this study takes 3 minutes. This protocol consists of normal breathing without monitoring respiration rate. Then, subjects are requested to increase the effort towards deep breathing. After reaching maximal breathing effort, at every 10s, HV is marked using marker and this is continued up to 3min duration. 3 min duration after HV is considered as PHV EEG. This process again took another 3 min.

Photic stimulation is applied after PHV using White Lux light at various frequencies namely 1 Hz, 3 Hz, 5 Hz, and 7 Hz. The photic driving frequencies are activated one by one consecutively. Every onset of photic stimulus is marked with a marker while recording. The entire EEG recording takes duration nearer from 8 to 12 min, because it depends upon the subject’s cooperation. Figure 3.2 shows the screen shot of the actual EEG recording for both normal and SCH subjects using Brain Tech Clarity equipment where Figure 3.2 (a) shows EEG for a normal and Figure 3.2 (b) shows for a SCH subject.

In Figure 3.2, the data acquisition tools like filter parameters, montage selection, sensitivity and the other data analysis tools like events, brain maps, and print etc., are given in the top of the recording screen, time is indicated at the bottom of the screen after onset of EEG recording. The left
Figure 3.2  Actual EEG recorded (a) EEG of Normal subject b) EEG of SCH subject
extreme bar indicates the electrode positions for the montage chosen and right extreme bar has indications about whether the electrode positions are in contact with the scalp or not, whether connected to low and high cut off filter frequencies or not. In a display window, 10s recording signal can be viewed in both online and offline.

3.5 EEG RECORDING DURING MENTAL TASK

Since this study protocol includes EEG signal analysis during mental activity, it is necessary to evoke cognitive process through stimulus. Determination of infrequent target performs more neural activity. The most commonly used oddball paradigm makes the subject to identify the infrequent target stimulus from the frequent non target stimulus. There are two types of oddball paradigm called auditory odd ball task and visual odd ball task. In a standard visual odd ball task the ‘squares’ are non target and ‘circles’ are target (Scott Huettel & Gregory McCarthy 2004). Whenever circle appears on the screen, either the subject has to respond or count mentally. The probability of occurrence of non target is higher than that of target. In an auditory odd ball task, the subjects should respond for the rare target tone inserted in a series of non target tones.

Many studies widely used either auditory oddball or visual oddball paradigm to analyze cognitive processes for SCH (Winterer et al. 2004; Bahar Guntekin et al. 2013). This research uses visual odd ball paradigm, because the SCH subjects who are involved in this study speak different languages and make them to understand about auditory stimulus may be difficult. Hence, visual oddball task is chosen in this research. So two modified visual oddball paradigms are specially designed to stimulate the mental activity, and they are named as Stimulus1 and Stimulus2 respectively. The reason for choosing two different modified odd ball paradigms are to observe the EEG changes due to different types of mental activity.
3.5.1 Modified Odd Ball Paradigm-Stimulus1

It is supposed to fix one target and set of non target things to design a stimulus. As suggested by the visual standard odd ball paradigm, the target picture must be odd one out from the set of non target pictures (Scott Huettel & Gregory McCarthy 2004). In this design, some group of images like sceneries, animals, flowers, transport vehicles, birds and baby pictures are considered. Compared to other images, baby picture comes under the category of human and helps the subject to easily differentiate from non target pictures. Hence, in this research, it is selected as a target and the remaining groups of images are treated as non target pictures which are selected from sceneries, animals, flowers, transport vehicles and birds.

![Figure 3.3 Target and non target pictures used for Stimulus1](image)

The designed modified odd ball paradigm with these pictures is called hereafter as Stimulus1. It is designed as follows. One run is said to be a display of one target picture along with 5 non target pictures which are displayed with 500ms and 750ms durations respectively. The mental activity
introduced in this type of paradigm is, how fast the subject recognizes the target picture. Therefore, duration for target picture is always less than the non target picture in our design. The probability of occurrence of target picture is 1/6. In a run, set of non target and target images are fixed. Figure 3.3 shows the list of pictures used for each of the runs.

For example, for the run1, sceneries are used as a non target pictures and a baby picture is used as target picture. Then when the paradigm moves to the run2, the animals are chosen as non target pictures. Similarly the remaining three runs are designed using the images shown in Figure 3.3. Every time when a run is repeated, the occurrence of the target picture is random. There are totally five runs in this paradigm and each run is repeated five times. The pictures are displayed with windows media player.

3.5.2 Modified Odd Ball Paradigm-Stimulus 2

In order to provide different mental activity, another stimulus is designed based on the same odd ball task. This modified oddball task hereafter is called Stimulus2. Here also, the baby picture is considered as a target image and set of flower images are used for non-target pictures. The pictures used in stimulus 2 are shown in Figure 3.4. Here there is only one run but it is repeated 9 times. Each time one baby picture, and 6 flower pictures are displayed in a defined order with equal time duration of 1s and the pictures are also slightly bigger in size compared to stimulus 1 to make the pictures much clear. A blank screen is displayed with 0.75s duration when the run is repeated. In this type of stimulus, a run is repeated 9 times with same set of target and non target pictures. This interval is necessary to indicate the beginning of the run. The probability of occurrence of target picture is 1/7. Here also pictures are displayed with windows media player.
Stimulus1 does not require any time interval between the runs because in every run, set of non target pictures are changed which can be an indicator for the beginning of a new run. There are two differences between the two stimuli designed. In stimulus1, the occurrences of target pictures are random which is not predictable and no time interval is maintained between the two runs. In stimulus2, target picture occurrence is predictable and time interval is maintained when each run is repeated. When stimulus1 and stimulus2 are shown to the subject, he or she has to count the number of target pictures whenever it appears on the monitor. Stimulus1, having 5 runs with each run having different non target pictures, this will be requiring more mental activity when compared to stimulus2 condition.

Here the onset of stimulus is not synchronized with EEG recording. The reasons are as follows. The conventional EEG is normally recorded for SCH subjects. Normally the standard EEG recording system used for such conventional recording will have synchronization of the PS with EEG recording. But in this study, the main objectives are to analyze EEG being recorded in the conventional conditions namely eyes closed, eyes opened, HV, PHV, photic stimulation as well as during mental activity being performed by the subject. This is performed by giving two types of stimulus namely stimulus1 and stimulus2. If we are interested in EP or ERP during
this mental activity, synchronization of the stimulus with EEG is essential wherein the EEG recording system should have a provision to store the two stimuli in it and synchronize them with EEG recording for EP or ERP analysis. In such a case, the complexity of the EEG system and hence the cost is increased when compared to the conventional standard EEG recording system.

To reduce such complexity and cost of analysis, it is decided to use the conventional standard EEG system itself for recording EEG during stimuli as we are making the subject involved in some mental activity compared to conventional EEG recording conditions. This is being tried as a novel method here to analyze EEG of subjects. Thus the period of EEG generated by the application of stimuli is marked manually and this raw EEG data will be analyzed to see whether it has features distinguishing the SCH subjects from normal subjects. The information about the target and the nontarget pictures are explained and shown to the subjects before recording. The subject is instructed to mentally count the number of target pictures during the stimulus presentation. The recording consists of conventional EEG recording followed by recording of EEG during stimulus presentation. The above-described two types of stimuli are displayed one after another on the 15-inch computer screen (color monitor) located at 1.5m distance from the subjects. The distance is chosen such that the subjects can clearly see the pictures displayed on the monitor.

### 3.6 PRE PROCESSING

This section explains about various pre processing techniques involved in removal of artifact from EEG. In general EEG is contaminated by other physiological signals and other artifacts. Artifacts from the other physiological signals are called physiologic artifacts and artifact from outside the human body is termed as other artifact. Other artifact such as 50Hz interference from the power supply is removed using notch filter during EEG
acquisition. Physiological artifact such as movements of the body and artifact due to sweat are rejected by visually inspecting data. Hence, chances of artifact arrived in EEG may be due to eye blink and eye ball movement. Eye blink amplitudes are generally 5-10 times higher than the EEG and its duration is about 400 ms. There are varieties of time and frequency domain methods available to remove eye blink from EEG. Such methods may require EOG as reference signal and there is a possibility of losing information contained in EEG data while removing eye blink artifact.

ICA becomes popular method to remove eye blink artifact without losing information in the underlying EEG signal. It can extract both eye blink and eye ball movement component from the EEG signal. Second order source separation Algorithm for Multiple Unknown Source Extraction (AMUSE) and Second Order Blind Identification (SOBI) algorithms are basic blind source separation algorithms for removal of artifact and it is completely based on the eigen value of the auto covariance matrix. Both methods use multiple step auto covariance process and it is also proven that AMUSE can perform equally well like SOBI (SaraTaskinen et al. 2012). AMUSE uses very simple unmixing matrix (identity) and more suitable for unknown source signal, this algorithm is considered to remove eye blink artifact.

Eye blink artifact present in EEG is shown in Figure 3.5 (a) and (b). Figure 3.5 (a) is the eye blink affected EEG of normal subject whereas Figure 3.5 (b) for SCH subject. The actual recorded EEG contaminated with eye blink artifact for both normal and SCH are shown in Figure 3.5. The eye blink is indicated with red circle. It is seen from the figure that the eye blinks are dominant in the frontal lobe electrodes F_P2, F_4, F_8, F_P1, F_3 and F_7. In order to remove such artifact without losing the actual information contained, the efficient blind source separation technique namely Independent Component Analysis (ICA) is used (Seungjin Choi et al. 2005). ICA LAB (ICA laboratory) is used to perform this.
Figure 3.5 (Continued)
Figure 3.5 Eye blink affected EEG (a) For Normal subject (b) For SCH subject
ICALAB is a package designed for blind source separation. It has many efficient algorithms such as higher order statistics, blind source separation, linear prediction, and blind signal extraction etc. It is a widely used technique to separate the linearly mixed source.

\[ X = AS \]  

(3.1)

where \( X \) is the observed data in the original space, \( S \) is the source activity and \( A \) is the weight matrix that contains mixing coefficient. After estimating mixing coefficient using ICA algorithm, the unmixing matrix becomes \( W = A^{-1} \), and the separated signal \( y \) is achieved by

\[ y = WX \]  

(3.2)

Estimating mixing coefficient “A” involves many algorithms, but AMUSE is used here, because it allows the components to be automatically ordered in decreasing order, the estimated components are less complex, and it is recommended for unknown source signal (Seungjin Choi et al. 2005). The procedure involved to implement this algorithm is shown below (http://www.bsp.brain.riken.jp/ICALAB/ICALAB SignalProc/).

For the purpose of illustration, 10s epoch of EEG data during stimulus presentation is taken and it is loaded into ICALAB. The signal sampling frequency should be uploaded.

- **Pre processing**- The first step of ICA algorithm begins with whitening data, in order to remove the correlations between the source and noise. Whitening is done by using identity matrix. Implementation of algorithm generally begins after pre-processing. This will improve the faster convergence of the results. (Seungjin Choi et al. 2005).

- **Selection of Channels**- Before execution of specific algorithm, the number of channels is to be selected for noise removal algorithm. For illustration purpose, EEG of one normal and SCH subject is loaded in ICALAB and are shown in Figure 3.6 and 3.7. The ICALAB window
shows 10s length EEG signal of normal subject. The recording has only 16 channels of which the first 10 are shown in Figure 3.6 (a) and the remaining 6 are shown in Figure 3.6 (b) as the last 6 traces. The top 4 traces of Figure 3.6 (a) are repeated as the first 4 traces in Figure 3.6 (b).

Figure 3.6 Eye blink affected normal subject EEG loaded into ICALAB
(a) Channels F_P2, F_4, C_4, P_4, F_8, T_4, T_6, O_2, F_P1 & F_3
(b) Channels T_6, O_2, F_P1, F_3, C_3, P_3, F_7, T_3, T_5 & O_1
Figure 3.7 (Continued)
Figure 3.7 Eye blink affected SCH subject EEG loaded into ICALAB (a) Channels Fp2, F4, C4, P4, F8, T4, T6, O2, Fp1 & F3
(b) Channels T6, O2, Fp1, F3, C3, P3, F7, T3, T5 & O1
as the ICA LAB window allows only 10 channels to be loaded. Similarly Figure 3.7 is shown for SCH. This window is mainly used to select the number of channels to be included for removal of eye blink by clicking the select channels bar in the bottom of the screen. Always number of extracted components will be smaller than the number of channels (Yaquab & Suresh 2018). Hence all channels are included in this work for execution of the algorithm in order to retain the information contained in the actual EEG signal.

- **Decomposition of multi-variable signals into independent components** - To extract the independent components, AMUSE algorithm is selected. This algorithm is performed by two basic steps as follows.

  - **First step:** \(x(n)\) is the 10s EEG length data sequence. The standard pre whitening process transformation

    \[
    x_1(n) = Qx(n) \tag{3.3}
    \]

    - Determination of \(Q\) - The covariance matrix of \(x(n)\) is obtained which is

    \[
    R_x = E[x(n)x^T(n)] \tag{3.4}
    \]

    SVD is applied to the covariance matrix

    \[
    R_x = V \Lambda V^T \tag{3.5}
    \]

    Then \(R_x^{-1/2}\) is determined using Equation (3.6)

    \[
    R_x^{-1/2} = (V \Lambda V^T)^{-1/2} = Q \tag{3.6}
    \]

    Finally, \(x_1(n)\) is obtained using Equation (3.3)
• **Second Step:** The delayed covariance matrix is obtained for the \( x_1(n) \) which is found using
\[
R_{x_1} = E[x_1(n)x_1^T(n - 1)]
\] (3.7)

- Then SVD is performed for \( R_{x_1} \) using Equation (3.8).
\[
R_{x_1} = U \Sigma V^T
\] (3.8)

At last the unmixing matrix \( W \) can be calculated using Equation (3.9).
\[
W = U^T V
\] (3.9)

After determining this unmixing matrix \( W \), the independent components can be separated (Seungjin Choi et al. 2005).

- **Extraction and removal of undesirable artifacts and interference**-
  This can be implemented by performing deflation. The extracted independent components are displayed in a new window, the moment deflation started. The unwanted independent components can be discarded as follows. Figure 3.8 and 3.9 correspond to the independent components of the Figure 3.6 and 3.7. In Figure 3.8 (a) and 3.9 (a) shown below, the eye blink artifact becomes the first independent component when the algorithm is executed. The remaining six components are shown in Figure 3.8 (b) and 3.9 (b) for both subjects. Then the deflation is carried out with the remaining components. The components whichever are to be removed can be deselected and thus it will be excluded during reconstruction.

- **Reconstruction of signal after removal of artifact**- After performing deflation, the signal is reconstructed using the remaining components as mentioned in the Equation (3.2). The screen shot of the eye blink...
artifact removed EEG signal is shown in Figure 3.10 and 3.11. Signal to Intereference ratio (SIR) of the mixing matrix is a criterion used to estimate the performance of the algorithm (Ali Al saegh 2015). It is a ratio of the signal power of the estimated independant component to the total power of the interfering signal. It is calculated for each channel and the mean SIR is considered by using the Equation (3.10).

\[
SIR = \frac{1}{N} \sum_{i=1}^{N} \left( \sum_{j=1}^{N} \frac{|P_{ij}|}{\max_{j} |P_{ij}|} - 1 \right)
\]  

(3.10)

where \( N \) is the number of sources. The result of the SIR is expressed in decibel (dB).

By recalling the Equation (3.1) and (3.2), the signal can be separated perfectly, if the unmixing matrix \( W \) is equal to \( A^{-1} \). The term \( P \) in the Equation (3.9) represents \( P = WA \). If \( W = A^{-1} \) then the signal is separated perfectly as per Equation (3.2). Thus \( P \) becomes 1, then \( SIR = 0 \), which means perfect independent component estimation. Actually, the estimated independent component found should be equal to the interfering signal (eye blink) for better extraction. Thus SIR should be very less for better reconstruction.

In this work there are totally 81 signals processed for removing eye blink. Number of eye blinks in a given EEG epoch varies from subject to subject and hence the SIR values vary from signal to signal. However, the maximum value of SIR for reconstructed signal is found to be 8.5251 dB when the entire 81 subjects eye blink affected EEG data set is processed. Lower SIR is the better separation of eye blink, thus this 8.5251 dB value indicates that the eye blink is removed effectively with retention of actual
(a)

Figure 3.8 (Continued)
Figure 3.8 Estimated independent components for normal subjects (a) 1 to 10 ICA components (b) 7 to 16 ICA components
Figure 3.9 (Continued)
Figure 3.9 Estimated independent component for SCH subject (a) 1 to 10 ICA components (b) 7 to 16 ICA components
Figure 3.10  (Continued)
Figure 3.10 Reconstructed signals for normal subject (a) $F_{P2}, F_4, C_4, P_4, F_8, T_4, T_6, O_2, F_{P1}$ & $F_3$ Channels (b) $T_6, O_2, F_{P1}, F_3, C_3, P_3, F_7, T_3, T_5$ & $O_1$ Channels
Figure 3.11 (Continued)
Figure 3.11 Reconstructed signals for SCH subject (a) FP2, F4, C4, P4, F8, T4, T6, O2, FP1 & F3 Channels  
(b) Channels T6, O2, FP1, F3, C3, P3, F7, T3, T5 & O1
information contained in that particular epoch. Eye blink artifact removal is also carried out using SOBI algorithm for testing purpose. The performance of the AMUSE algorithm is improved and it is approximately equated to SOBI by adjusting the delay parameter (in ICALAB). This is also one of the reasons for choosing AMUSE. The artifact removed EEG is considered for further processing. There is a possibility of changes in the signal amplitude level, while performing this algorithm. Hence, after this process the signal is normalized for further processing to be carried out in this research.

3.7 SUMMARY

This chapter begins with the explanation about the selection of subjects for this study. In this study 81 subjects are selected, among them 52 are SCH and 29 are normal subjects. In order to stimulate mental activity, two stimuli called stimulus1 and stimulus2 are designed based on the visual odd ball paradigm. The EEG recording protocol is formed. EEG is recorded from 81 subjects as per the protocol framed. The conventional EEG recording protocol includes namely resting, HV, PHV and PS conditions where resting condition is considered as eyes closed EEG recording before HV starts.

In continuation with the conventional recording, EEG is recorded during presentation of stimulus1 and stimulus2. The recorded EEG is contaminated with eye blink artifact. Therefore, pre-processing technique has been performed to remove eye blink artifact. Thus it is removed from EEG using AMUSE algorithm in ICA LAB. The eye blink removed EEG is validated using SIR value of the mixing matrix and reconstructed signal.

As explained in the proposed work shown in Figure 2.1, feature extraction from the pre-processed EEG is the next step of this research. The purpose of the next chapter is to find such features which can provide information about differences between normal and SCH subjects.