# Transdiagnostic Investigation of Psychiatric Disorders with Transcranial Magnetic Stimulation


A thesis submitted towards partial fulfilment of BS-MS Dual Degree Programme

by

**Aboli Ektare (20141075)**

For the study conducted under the guidance of

**DR. A Shyam Sundar**

at the

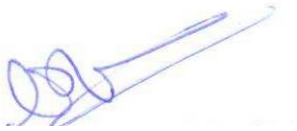
**Department of Psychiatry,**

**National Institute of Mental Health and Neuroscience, Bangalore**

# Certificate

This is to certify that this dissertation entitled "**Transdiagnostic Investigation of Psychiatric Disorders with Transcranial Magnetic Stimulation**" towards the partial fulfilment of the BS-MS dual degree programme at the Indian Institute of Science Education and Research, Pune represents study/work carried out by **Aboli Ektare**, at **National Institute of Mental Health and Neuroscience, Bangalore** under the supervision of **Dr. A Shyam Sundar, Department of Psychiatry, National Institute of Mental Health and Neuroscience, Bangalore** during the academic year **2018-19**.

Signature of Student



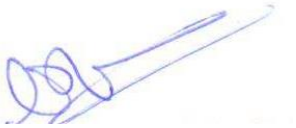
Signature of Supervisor

Date: 20/03/2019

# Declaration

I hereby declare that the matter embodied in the report entitled "**Transdiagnostic Investigation of Psychiatric Disorders with Transcranial Magnetic Stimulation**" are the results of the work carried out by me at the **Department of Psychiatry, National Institute of Mental Health and Neuroscience, Bangalore** under the supervision of **Dr A Shyam Sundar**, and the same has not been submitted elsewhere for any other degree.

Signature of Student



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## ABSTRACT

Transcranial Magnetic Stimulation (TMS) is a non-invasive investigational tool that allows scientists to stimulate cortical areas of interest, which assists in studying the neurophysiology of the brain with temporal and spatial precision. Often TMS is administered to the human primary motor cortex as the activity in surface muscles like First Dorsal Interosseous can be used to quantify the response of intracortical and corticospinal response to magnetic pulses. Experiments with quantification of muscle response to different types of pulses as well as different combinations of magnetic pulse intensity and inter-pulse interval have yielded multiple TMS pulse paradigms that are dependent on excitatory and inhibitory mechanisms, modulated by different neurotransmitters such as Glutamate and Gamma-Aminobutyric Acid (GABA). We used three excitatory and two inhibitory paradigms to assess motor cortical response in patients suffering from various psychiatric disorders including Major Depressive Disorder (MDD), Schizophrenia, Bipolar Affective Disorder (BPAD) Mania, Obsessive-Compulsive Disorder (OCD) as well as Healthy Volunteers and First-degree relatives of BPAD Mania patients. Motor cortical excitability measures corresponding to $GABA_B$ are lower in patients with OCD and MDD, while they are higher in BPAD Mania patients. Patients with OCD and Depression also display enhanced motor cortical excitability when compared to patients with other disorders as well as healthy volunteers. Altogether, our findings indicate a deficiency in GABAergic mechanisms in OCD and MDD. The functioning of various neurotransmitter systems can be studied indirectly through TMS, which may help identify potential biomarkers for specific psychiatric conditions.

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**ACKNOWLEDGEMENTS**

First and foremost, I would like to express my sincere gratitude to Dr A. Shyam Sundar who not only gave me the opportunity to work in NIMHANS providing exposure to the research work underway in the institute but also has been a source of unwavering support and encouragement. During the course of the project, he gave me the freedom to pursue my interests and provided me with the environment where I could put forth any questions and ideas about the experimental designs. I am thankful to him for bearing with my unfamiliarity toward the techniques in the beginning and also for supplying me with all the necessary guidance during times of uncertainty.

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A special thanks to my family and friends for having faith in me and ensuring that I stay on the right path towards my goals. I am also grateful for the healthy volunteers, the patients as well as their relatives who were kind enough to let us do the assessments on them.

Lastly, I would like to thank the Department of Psychiatry, NIMHANS for providing me access to TMS Lab and IISER, Pune and DST-INSPIRE for funding this project.

The submitted thesis proposal, as well as the mid-term report, was more focused on the modulation of motor cortical plasticity using Theta Burst Stimulation as a treatment on depression patients and healthy controls. This study could not be presented as my MS thesis for the reasons mentioned below:

1. Post-approval from NIMHANS Ethics Committee, the protocol was optimized for time and efficiency with the help of pilot studies. Till mid-term only 3 patients and 3 healthy controls were recruited for the study. And till February 2019, 5 patients and 5 healthy controls were recruited. Which was not enough to make any conclusions or for identification of any existing patterns.
2. Since the student is a non-medical person, direct patient contact was strictly out of bounds as per NIMHANS policy. Recruitment was only on a referral basis and was also dependent on the number of consultations for depression in the Out Patient Department. These factors were out of the student's and supervisor's control.
3. The approved protocol for the original thesis topic contained certain inclusion and exclusion criteria which were meant to be strictly followed during the recruitment of subjects for assessment. One of the criteria was that only those patients with Major Depressive Disorder (MDD) who are referred for TMS treatment will be considered as potential recruitment candidates. Other criteria involved the definite primary diagnosis of MDD followed by a minimum score on the Hamilton Depression Rating Scale which some patients referred for TMS treatment did not qualify and hence were not taken for the study.

During the time, there was another study being conducted on the data already collected by previous as well as ongoing studies in the same lab using the same techniques mentioned in the mid-term report. The student was not part of the data collection or subject recruitment procedure. Raw data files from these studies were used by the student for processing and analysis.

## INTRODUCTION

Transcranial Magnetic Stimulation (TMS) is a neurophysiologic technique introduced in 1985 (Barker et al., 1985). Transcranial Magnetic Stimulation induces current on the basis of Faraday 's principle of Electromagnetic Induction. Time-varying electric current passing through the copper coil generates a magnetic field which then induces an electric current in the conducting surface (Faraday M, 1831/1965) that comes in contact with the coil, i.e. Brain tissue. Unlike electrical stimulation of nervous tissue via electrodes, TMS works indirectly by means of electromagnetic induction causing charge to move across cellular membrane giving rise to transmembrane potential. If the power of the magnetic field is high enough, it can cause neuronal depolarization which passes through the axons as Action Potential. TMS is capable of providing highly focal stimulations and thus helps in identifying the localization of function in the brain.

TMS allows scientists to stimulate brain non-invasively in alert and awake adult human subjects. It has been used to study intracortical and cortico-cortical interactions, to assess the causal relationship between brain activity and behaviour as well as to establish neurophysiologic substrates of various psychiatric and neurophysiologic illnesses (Pascual-Leone et al., 2002, Horvath et al., 2011). More specifically single TMS pulses are used to induce Motor Evoked Potential (MEPs) in peripheral muscles, which is measured with Electromyography (EMG). The MEP amplitude is a direct measure of corticospinal excitability (Rotenberg et al., 2014). With the development of technology and experimental designs, new paradigms have been in use to assess different aspects of the neuronal connectivity such as facilitation (potentiation) and inhibition in healthy volunteers as well as patients with neurological and psychiatric disorders.

Psychiatric disorders (depression, schizophrenia) are among the leading causes of disabilities in the world (Harvey et al., 2013). The global estimation of people suffering from Depression is 300 million, from Bipolar Affective Disorder is over 60 million and that of Schizophrenia and other psychoses is around 23 million ((World Health Organization/Fact Sheet/Mental Disorders, 2018). Diagnosis of psychiatric disorders is based on the combination of subjective changes in cognition, mood, perceptual and behavioural changes as reported by patients and their relatives. There are guidelines designated by the World Health Organization for diagnosis which are formulated in the

International Statistical Classification of Diseases and Related Health Problems, 10th Edition (ICD-10), chapter V of which is used by psychiatrists for diagnosis of psychiatric conditions. For each of the disorders, a description of the main clinical features is provided along with important but less specific features. Diagnostic guidelines are then specified for most cases indicating the number and balance of symptoms required to make a confident diagnosis. There also is certain flexibility for diagnostic decisions in clinical scenarios where the preliminary/cursory diagnosis has to be made before a complete picture emerges.

Disorder is defined as a set of clinically recognized symptoms or behaviour associated most cases with distress and which interferes with personal functions. Psychiatric disorders are defined empirically based on their presenting symptoms and not on their underlying pathophysiology. Various techniques, such as neuroimaging, electrophysiological recordings etc. have been employed to study the pathophysiology of these conditions. There is evidence to suggest that different psychiatric conditions may be caused by different neurotransmitter system and brain network abnormalities (George and Belmaker, 2007). In the current study, we planned to study the involvement of different neurotransmitter systems in different psychiatric disorders through TMS related cortical excitability measures.

As part of the study, TMS raw data of subjects from ongoing projects and the ones from previous studies in the TMS lab at NIMHANS was included in the processing and analysis. The studies included the following psychiatric disorders – Major Depressive Disorder (MDD) (Ongoing), Schizophrenia (Mehta et al.,2014), Bipolar Affective Disorder (BPAD) Mania (Basavaraju et al.,2017) and Obsessive-Compulsive Disorder (OCD) (Arumugham et al.,2018). The subject population also included healthy volunteers (Mehta et al., 2014; and from ongoing Depression studies) and First-degree relatives (at high risk of developing mania) of patients of BPAD (Basavaraju et al., 2017). The individual studies were conducted with a focus on respective psychiatric disorders in the TMS lab between 2011-2018. Patient recruitment was also done during the aforementioned studies. Raw data files were processed by the student including individual measurements of certain TMS parameters in the software (Signal for Windows) and then analyzed.

The ICD-10 guidelines for the diagnosis of Schizophrenia, BPAD Mania, MDD and OCD are stated as –

"**Schizophrenia – F20**

General symptoms – (a) Thought echo, thought insertion or withdrawal and thought broadcasting

(b)Delusions of control, influence, or passivity, clearly referred to body or limb movements or specific thoughts, actions, or sensations; delusional perception

(c)Hallucinatory voices giving a running commentary on the patient's behaviour, or discussing the patient among themselves, or other types of hallucinatory voices coming from some part of the body;

(d)Persistent delusions of other kinds that are culturally inappropriate and completely impossible, such as religious or political identity, or superhuman powers and abilities (e.g. being able to control the weather, or being in communication with aliens from another world)

(e)Persistent hallucinations in any modality, when accompanied either by fleeting or half-formed delusions without clear affective content, or by persistent over-valued ideas, or when occurring every day for weeks or months on end

(f)Breaks or interpolations in the train of thought, resulting in incoherence or irrelevant speech, or neologisms

(g)Catatonic behaviour, such as excitement, posturing, or waxy flexibility, negativism, mutism, and stupor;

(h)"Negative" symptoms such as marked apathy, the paucity of speech, and blunting or the incongruity of emotional responses, usually resulting in social withdrawal and lowering of social performance; it must be clear that these are not due to depression or to neuroleptic medication

(i) A significant and consistent change in the overall quality of some aspects of personal behaviour, manifest as loss of interest, aimlessness, idleness, a self-absorbed attitude, and social withdrawal.

Diagnosis: The normal requirement for a diagnosis of schizophrenia is that a minimum of one very clear symptom (and usually two or more if less clear-cut) belonging to any one of the groups listed as (a) to (d) above, or symptoms from at least two of the groups

referred to as (e) to (h), should have been clearly present for most of the time during a period of 1 month or more. Conditions meeting such symptomatic requirements but of duration less than 1 month (whether treated or not) should be diagnosed in the first instance as acute schizophrenia-like psychotic disorder (F23.2) and reclassified as schizophrenia if the symptoms persist for longer periods.

**Bipolar Affective Disorder Mania – F31.2**

The clinical picture of mania is of a severe form with inflated self-esteem. Grandiose ideas may develop into delusions, and irritability and suspiciousness into delusions of persecution. In severe cases, grandiose or religious delusions of identity or role may be prominent, and flight of ideas and pressure of speech may result in the individual becoming incomprehensible. Severe and sustained physical activity and excitement may result in aggression or violence, and neglect of eating, drinking, and personal hygiene may result in dangerous states of dehydration and self-neglect.

Bipolar Affective Disorder is characterized by repeated episodes (i.e. at least two) in which the patient's mood and activity levels are significantly disturbed, this disturbance consisting on some occasions of an elevation of mood and increased energy and activity is termed as BPAD Mania. Manic Episodes usually begin abruptly and last for between 2 weeks and 4-5 months (median duration of about 4 months) The frequency of episodes and the pattern of remissions and relapses are both very variable, though remissions tend to get shorter as time goes on and depressions to become commoner and longer lasting after middle age.

**Major Depressive Disorder – F32.2**

In a typical depressive episode, the individual suffers from depressed mood, loss of enjoyment and interest, and reduced energy leading to fatiguability and diminished activity. Other common symptoms include (a)reduced concentration and attention; (b)reduced self-esteem and self-confidence; (c)ideas of guilt and unworthiness (even in a mild type of episode); (d)bleak and pessimistic views of the future; (e)ideas or acts of self-harm or suicide; (f)disturbed sleep; (g)diminished appetite.

The subjects from included studies were of moderate to severe depression category. For this categorization, the patient should display at least two of the typical symptoms and at least 3 of the common symptoms for at least 2 weeks. In a severe depressive episode,

the sufferer usually shows considerable distress or agitation, unless retardation is a marked feature. Loss of self-esteem or feelings of uselessness or guilt are likely to be prominent, and suicide is a distinct danger in particularly severe cases. Due to the severity of symptoms in the latter case the diagnosis can be made after less than 2 weeks.

**Obsessive-Compulsive Disorder – F42**

For a definite diagnosis, obsessional symptoms or compulsive acts, or both, must be present on most days for at least 2 successive weeks and be a source of distress or interference with activities. The obsessional symptoms should have the following characteristics: (a) they must be recognized as the individual's own thoughts or impulses; (b) there must be at least one thought or act that is still resisted unsuccessfully, even though others may be present which the sufferer no longer resists; (c) the thought of carrying out the act must not in itself be pleasurable (simple relief of tension or anxiety is not regarded as pleasure in this sense); (d) the thoughts, images, or impulses must be unpleasantly repetitive." (Chapter V, ICD-10, WHO, 2016)

The diagnostic criteria and symptoms mentioned above are amongst the ones that happen occur most commonly. Although there are types in each category, the basic necessary symptoms are the aforementioned ones.

It is now widely accepted that psychiatric disorders have a biological basis (George and Belmaker, 2007), there is a need to look for definite biological markers in the disorders to improve the accuracy of diagnosis and treatment. Also, with the variability of symptom presentation as well as of the individual response to medication, definite and accurate clinical diagnosis in each case is a challenge for psychiatry.

TMS provides access to check excitability of corticospinal tract of the subject and hence the basic neurobiological response to a specific combination of stimuli. Hence it is considered a promising investigational tool along with other neuroscientific tools like ElectroEncephaloGraphy (EEG), Neuroimaging techniques like Magnetic Resonance Imaging (MRI), Positron Emission Tomography (PET) etc. as well as blood tests for various hormones, neurotransmitters, and neuropeptides (Rotenberg et al., 2014).

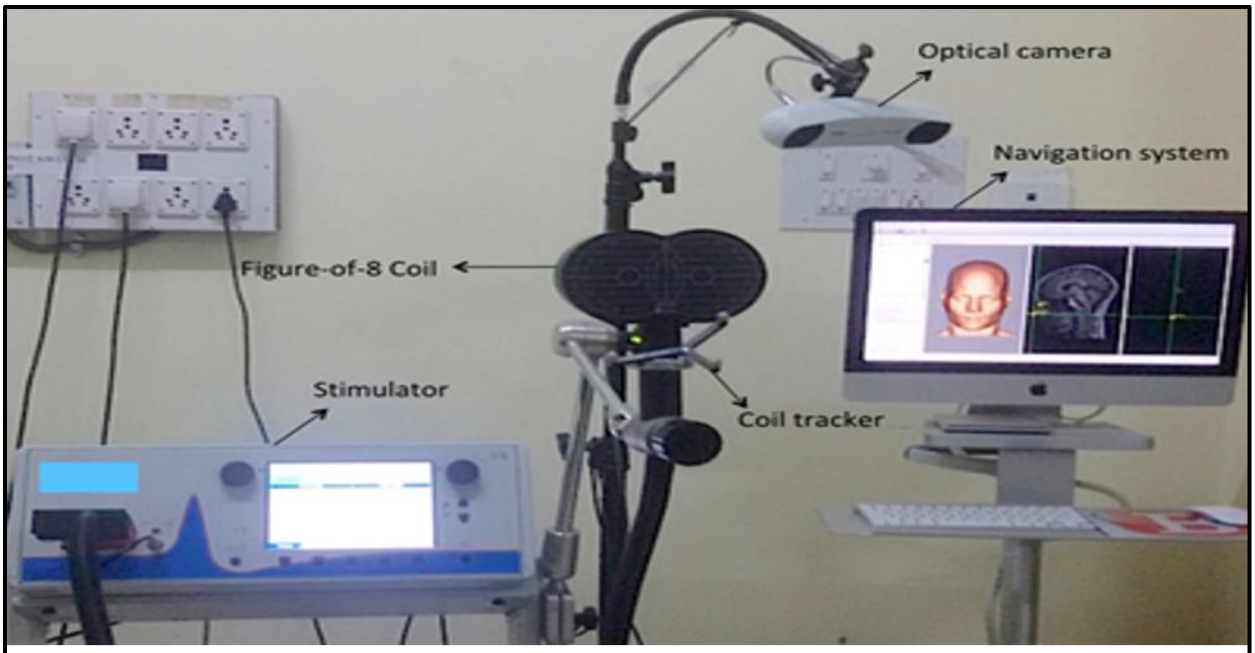


**Fig.1** – TMS setting with MRI guided neuronavigation system in TMS lab at NIMHANS, Bangalore.

Primary goal of TMS over primary motor cortex (M1) is to understand and assess motor cortical excitability which in people with psychiatric disorders can be affected by multiple factors such as age, gender, medication, severity of disorder, duration of illness, daily physical activity etc. All of the aforementioned sociodemographic data is not available for all the subjects included in the study and hence only age, gender and education are the parameters that have been considered during analysis. A major focus on the variability of TMS parameters between and within groups in order to understand the differences and similarities in them.

<u>The objectives of the study are as follows</u> –

1. To compare the motor cortical excitability parameters studied with Transcranial Magnetic Stimulation between patients with different psychiatric disorders.
2. To study the differences in motor cortical excitability parameters between patients with psychiatric disorders and healthy subject.

## MATERIALS AND METHODS

All the assessments were approved by the Ethics Committee, NIMHANS, Bangalore and performed in accordance with the TMS Safety Guidelines (Rossi et al., 2009) under the supervision of a medical doctor or psychiatrist. NIMHANS Ethics Committee approved informed consent was obtained from all participants. All the protocols were scientifically reviewed by the corresponding funding agency or the scientific committee at NIMHANS.

### Subjects –

A total of 416 human subjects including healthy volunteers, patients and relatives of patients underwent baseline Transcranial Magnetic Stimulation assessments between 2011-18 in TMS Lab at NIMHANS. This includes 130 Healthy Controls (HC), 100 patients suffering from moderate to severe Depression (Hamilton Depression scale rating >18), 90 patients with Schizophrenia (Positive and Negative Symptoms Scale rating between 30-130 i.e. mild to severe), 28 patients of BPAD Mania (Young Mania Rating Scale score >12), 30 first-degree relatives of BPAD patients (High Risk BPAD) (YMRS score ≤ 3) and 38 patients with Obsessive-Compulsive Disorder (OCD) (Yale-Brown Obsessive Compulsive Scale score ≥16).

### Assessment –

The assessments were done with TMS apparatus i.e., MagPro R30 with MagOption, developed by Mag-Venture (Farum, Denmark).

TMS machine has the following components (Rotenberg et al., 2014)–

1. **The Charging System** generates a time-varying electric current which grows to a peak and comes back to zero in<1ms which passes through the coil giving rise to a magnetic pulse.
2. **Energy Storage Capacitors** generate, store and discharge multiple energy pulses in rapid succession. This makes it possible to give two or more pulses with time interval of 1-3 ms between them.
3. **Energy Recovery Unit** allows for the apparatus to recharge post discharge.
4. **Thyristors** are electrical devices with the ability to switch large currents over a short period of time. It acts as a bridge between the stimulator and the coil transferring large amount (about 500J) of energy in 100ms.
5. **Pulse Shape Circuitry** gives the researcher a choice between different types of

pulses e.g. Monophasic pulse, Biphasic pulse, Twin pulses, Dual Pulses, Biphasic Bursts each with a characteristic shape and energy output. For investigational purposes, the shape of the electric and magnetic pulse is generally preferred to be of Biphasic (sine wave) type which has been found to give higher stimulation with low magnitude pulse as compared to the Monophasic pulse (Sommer et al., 2006; Di Lazzaro et al., 2001). Biphasic pulses generate full positive-zero-negative voltage oscillations which in turn causes a rapid directional shift in the current, initial and induced.

6. **Machine Display** shows the percentage of machine maximum output, realized di(current)/dt as well as the coil type and coil temperature. The machine has specialized circuits for temperature detection to prevent overheating of coils and skin burn. All machines are set to stop stimulation if the coil temperature reaches 41°C. There are settings available to change the machine output, pulse type, external trigger and sensitivity for Motor Evoked Potential during the assessment. (Mag-Venture User Guide)

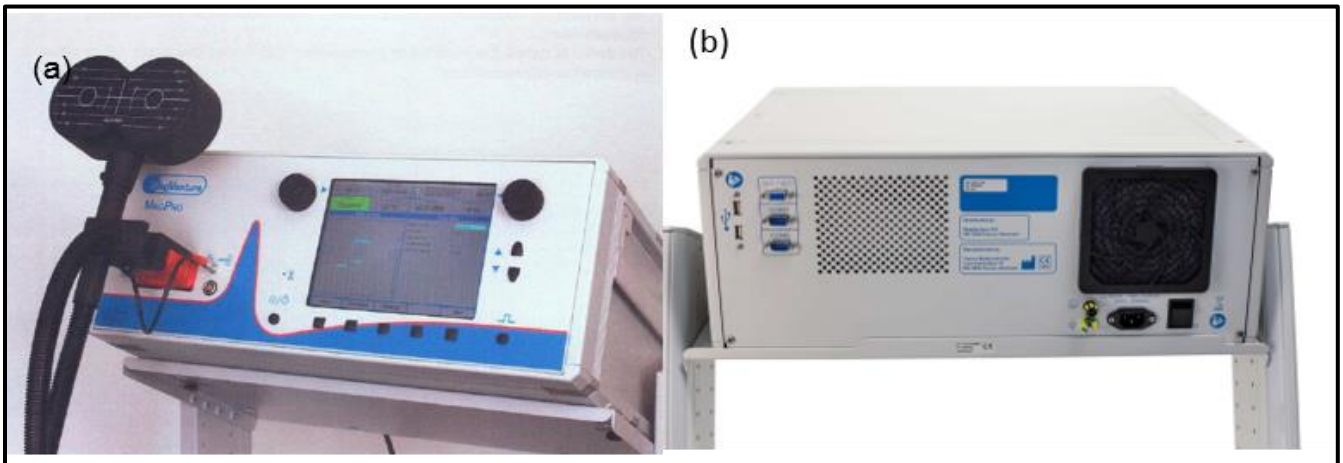


Fig.2 – TMS machine by Mag-Venture, Farum, Denmark.
Mag-Venture TMS machine with coil; b) Rear view of TMS machine. Panel available for MEP monitor attachment. ©2010 Tonica Elektronic A/S

7. **TMS Coil** used for the experiments (MC-B70) (Fig.3a) has two thick copper coils placed next to each other with the resultant shape of that of the figure of 8 or butterfly. The current flows through the copper coils in opposite directions resulting in the current vectors adding up in the centre which makes the magnetic field at the centre of the coil strongest (1.5-3Tesla) reducing exponentially with increasing distance. (Mag-Venture User Guide) The magnetic field at the centre penetrates 2.5-5 cm deep through the skull

into brain tissue. (Deng et al., 2012) Due to the shape of the TMS coil, the shape of the magnetic field at the centre is of an inverted cone with a magnetic field highest at the base i.e. cortex surface reducing exponentially toward the apex i.e. deeper brain structures (George and Belmaker, 2007).

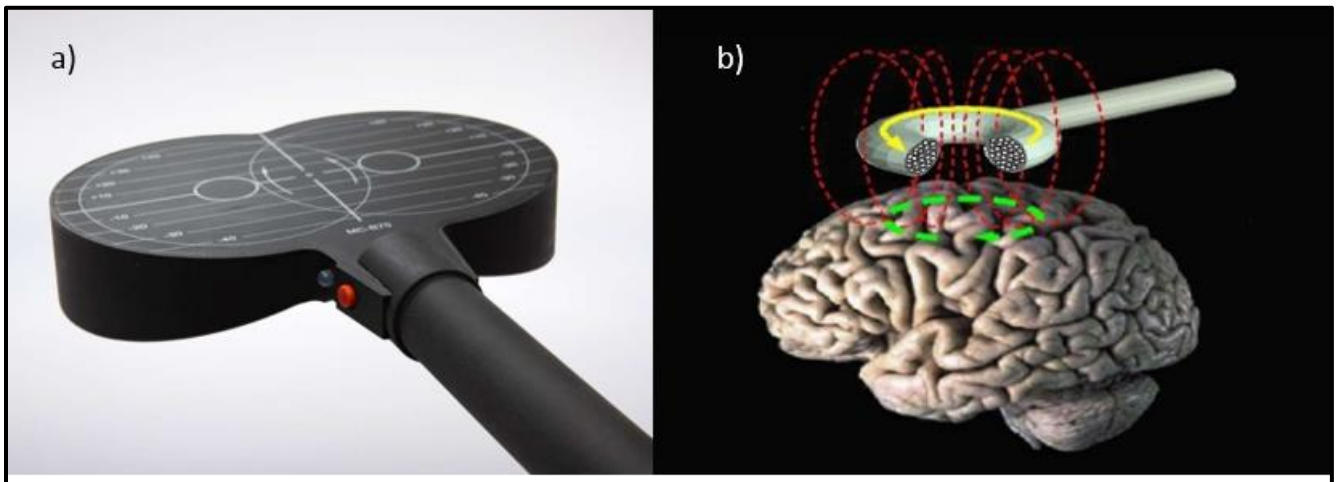


Fig.3 – TMS coil and current. a) MC-B70 coil by Mag-Venture. The direction of current flowing through the coil depicted, b) Direction of induced current opposite to the direction of initial current in the coil.

The coil is placed on the subject's head such that the direction of current flowing through the coil is parallel (hence magnetic field perpendicular) to the direction of neurons in the target region (Meyer et al., 1991) however opposite in direction to the initial current flow in the coil (Fig.3b; Hallett M, 2000; Kobayashi and Pascual-Leone, 2003).

8. **Electromyographic (EMG) Electrode** placement for quantification of primary motor cortex output is on First Dorsal Interosseous (FDI) muscle on right hand between thumb and forefinger. The passive electrodes have a coating of Ag/AgCl and electroconductive gel is applied on the electrode as well as target muscle so as to increase their sensitivity to the activity of the muscle underneath. There are three electrodes used during the experiment – one active electrode goes to the target muscle, the second electrode goes on the bone of either the thumb or the forefinger to measure and eliminate skin conductance, and the third electrode goes on the forearm tendon acting as noise cancelling electrode. The placements ensure that only muscle activity will be measured and no other aspects like skin/air conductance as well as movement in the rest of the arm (Groppa et al., 2012). When a pulse is given to the target tissue corresponding to FDI muscle, there is a visible twitch observed in forefinger or thumb for

the pulse of high intensity which is then picked up by the electrodes in the form of voltage change. (Rotenberg et al., 2014; Fig.4b)

9. **Signal Software** (4.0.7) developed by Cambridge Electronic Designs Ltd. (CED), Cambridge, England is used for detection and further processing of Motor Evoked Potential (MEPs) generated after the magnetic field is triggered/discharged from the stimulator over the primary motor cortex (M1). An MEP box acts as a bridge between R30 machine and CED box which converts analogue signals from the machine to digital data that the Signal software can process and present as a graph of voltage vs time (Fig.6). The amplitude of MEP generated is generally measured between 10-70ms after the trigger is given from the coil with the exception of one inhibitory parameter (long interval intracortical inhibition) (The CED Micro 1401 owners handbook, 2013; Signal for Windows, Version 4.07, 2010).

As for all other techniques, there are criteria which the participants have to pass in order to qualify for recruitment for assessments. As mentioned above, the TMS machine generates a magnetic field of the order of 1.5-3Tesla (MagVenture User Manual) and hence there are certain contraindications for it. Very rarely (<0.1%), the subjects may develop seizures. The risk of seizures is more in people with a history of seizures or other major brain pathology. Further, the magnetic pulse may overheat or displace any ferromagnetic substances in the vicinity. The presence of such substances in the body such as metallic implants for the treatment of fractures of the bones are contra-indications (Rossi et al., 2009).

These are made into a questionnaire, Transcranial Magnetic Stimulation Adult Safety Screening (TASS) (Rossi et al., 2011) which has 13 questions as follows –

(1) Do you have epilepsy or have you ever had a convulsion or a seizure?

(2) Have you ever had a fainting spell or syncope? If yes, please describe on which occasion(s)?

(3) Have you ever had a head trauma that was diagnosed as a concussion or was associated with loss of consciousness?

(4) Do you have any hearing problems or ringing in your ears?

(5) Do you have cochlear implants?

(6) Are you pregnant or is there any chance that you might be?

(7) Do you have metal in the brain, skull or elsewhere in your body (e.g., splinters, fragments, clips, etc.)? If so, specify the type of metal.
(8) Do you have an implanted neurostimulator (e.g., DBS, epidural/subdural, VNS)?
(9) Do you have a cardiac pacemaker or intracardiac lines?
(10) Do you have a medication infusion device?
(11) Are you taking any medications? (please list)
(12) Did you ever undergo TMS in the past? If so, were there any problems.
(13) Did you ever undergo MRI in the past? If so, were there any problems.

These questions ensure the absence of any metallic instrument in the head area that might get heated or displaced due to the administration of the magnetic field. History of Seizures also disqualifies the subject as they would be prone to getting seizures during TMS on the motor cortex.

The process from trigger press of the coil to target muscle activation is as follows –
At the moment of pulse delivery, the capacitors release the stored energy and an intense time-varying electric current passes through the copper cables into the coils. The currents flowing in the opposite direction add up the changing electric fields and hence the induced magnetic fields at the centre of the coil. The coil is held at a 45° angle to the parasagittal plane of the brain in order to stimulate the parallel underlying neurons in the primary motor cortex (M1). It is also important to make sure that that centre of the coil is in direct contact with the scalp. This also ensures the least amount of impedance to the penetrating magnetic field before it reaches the cortex. This magnetic field produces eddy currents in the cortex opposite to the direction of currents in the coil in neurons parallel to the coil or with axons bending away from the direction of induced current resulting in their depolarization and generation of action potential (Hodgkin and Huxley, 1952; Nagarajan et al., 1993; Tofts PS, 1990) in their respective axons (Fig.4a). These neurons then activate corticospinal motor neurons as well as interneurons via trans-synaptic mechanisms resulting in the contralateral target muscle twitch (Fig.5b; Lefaucheur et al., 2014). This twitch is then picked up by EMG electrodes and represented in Signal software as a graph of voltage vs time maintaining trigger at time point zero.

The parameters used in this study were all assessed at baseline (resting state). Although the individual studies had different protocols according to their respective goals, all baseline assessments were conducted in the same manner on the same stimulation machine with the same coil.

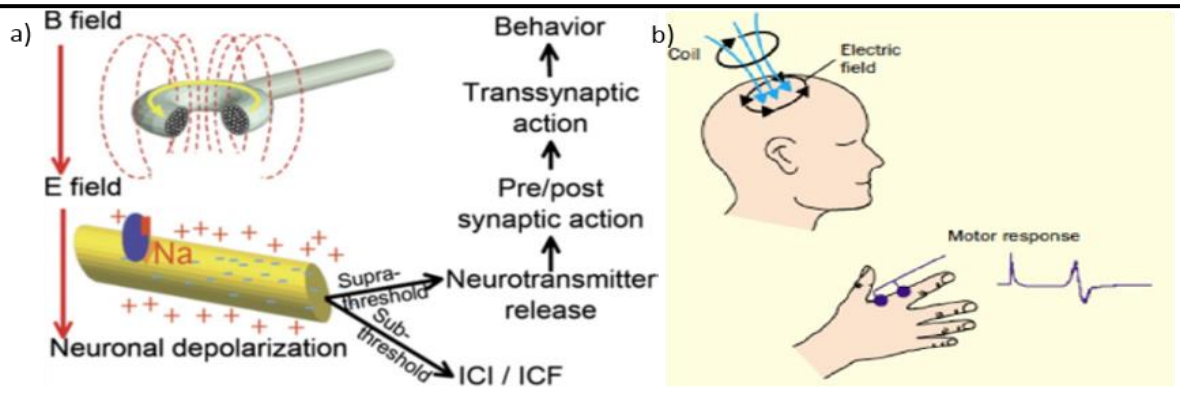


Fig.4 – TMS mechanism of action and response. a) Magnetic field from the coil gives rise to electric field causing neuronal depolarization resulting in transsynaptic actions gives rise to changes in behaviour of the target muscle.; b) Stimulation of cortical neurons causes activity in FDI which is picked up by EMG electrodes. (McClintock et al., 2011)

**Localization of Motor Hot Spot for FDI** – The FDI muscle representation on the Homunculus is localized with the help of 10-20 Electroencephalography (EEG) system (Fig.5a). The Nasion-Inion distance, as well as Inter tragal distance, is measured. The intersection of these two lines at their respective centres is termed as Cz (Fig.5a). The left motor cortex (contralateral to right-hand muscles) is localized around 5cm lateral and 1cm anterior on the left of Cz. After marking this point, trial and error method is used for identification of motor hotspot which generates the highest MEP amplitude at given machine output for right FDI muscle (Rothwell, 1997). Post localization, single pulse and paired-pulse paradigms are used for assessment of motor cortical excitability.

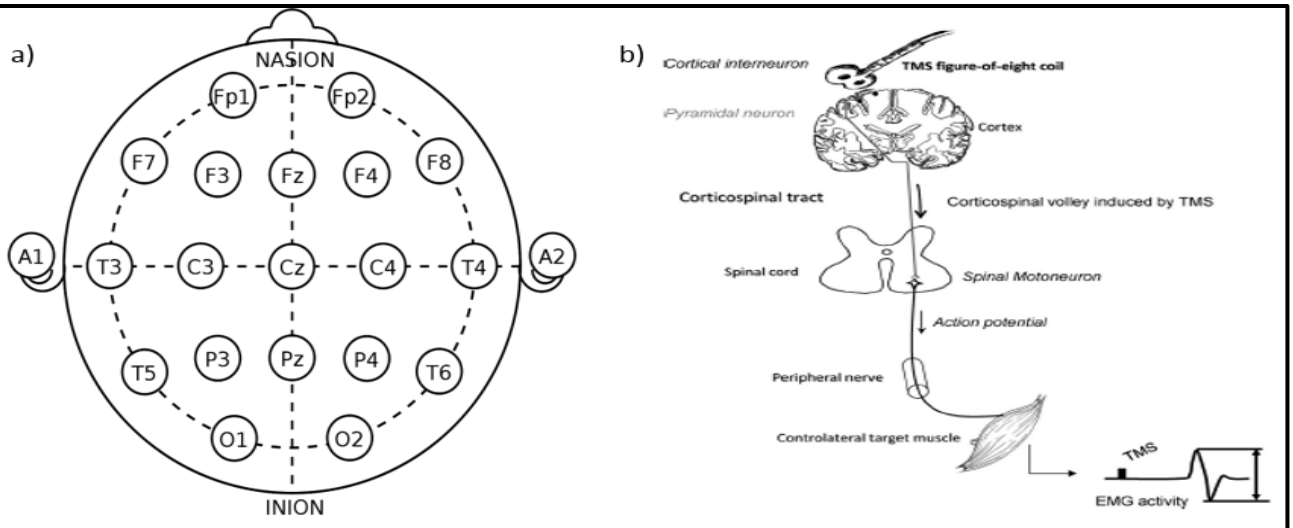


Fig.5- Target localization and stimulation. a) 10-20 EEG system for localization of Cz using nasion-inion distance and inter-tragal distance., b) Corticospinal conduction of stimulation from primary motor cortex to contralateral FDI muscle (Klomjai et al., 2015).

**Single Pulse Paradigms** –

1. Resting Motor Threshold – In TMS, the corticospinal excitability is determined by identification of Motor Threshold (MT). It is used to characterize an individual's corticospinal excitability for information regarding corticospinal path integrity for experimental as well as clinical purposes (Rotenberg et al., 2014). For assessments, it serves as the baseline measure used to define other single and paired-pulse paradigms. Measurement of MT when the muscle is at rest is called Resting Motor Threshold (RMT). RMT (Fig.6) is defined as the minimum stimulation intensity necessary to produce MEPs with a peak to peak amplitude of ≥50μV in 5 out of 10 consecutive single pulses (Boroojerdi et al., 2001; Hess et al., 1987). Each pulse is separated from the previous one by 3-5 seconds to avoid conditioned response to the sound of the TMS machine. After localization of the motor hotspot, the position and angle of the coil with respect to the scalp have to be maintained for consistency of results. Several studies suggest that RMT might reflect cortical excitability of the elements activated by TMS including ion-dependent neuronal membrane, cortico-cortical axons and their excitatory synaptic connections with corticospinal neurons (Chen et al., 1997). Blocking voltage-gated sodium and calcium channels has been shown to increase RMT whereas NMDA antagonists (Ketamine) have shown to decrease RMT values for individuals. GABAergic drugs do not seem to have any effect on the value of RMT. (Ziemann et al., 1996)

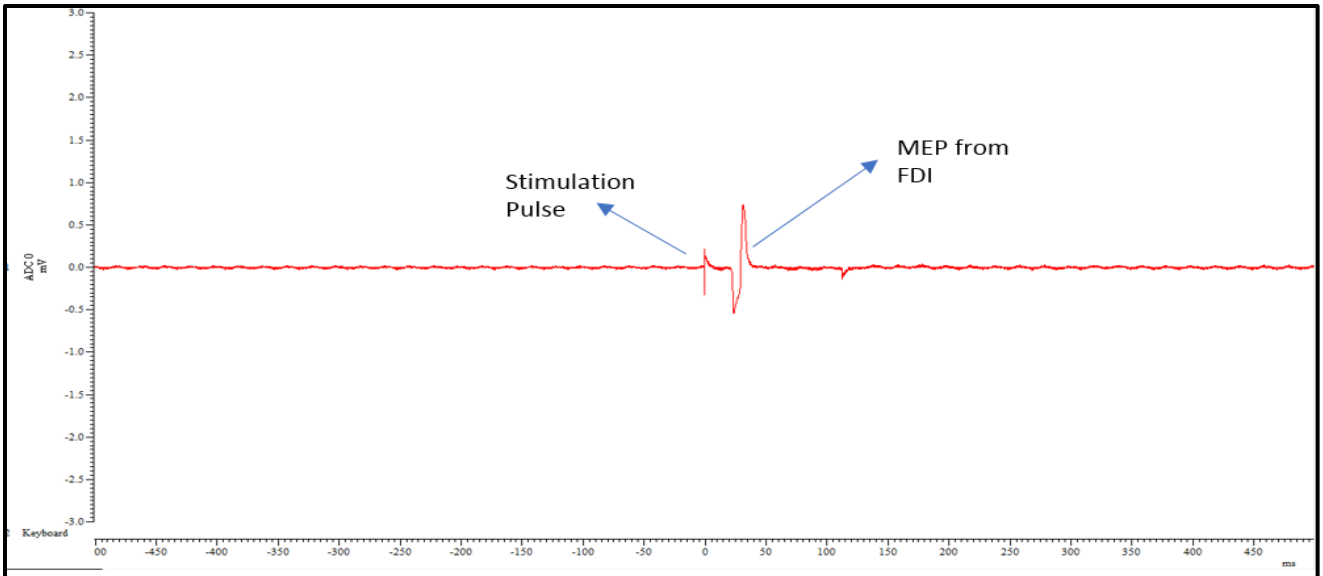


Fig.6 – Representation of Motor Threshold single pulse paradigm in Signal software. X-axis represents time in ms and Y-axis represents evoked potential in mV

2. Motor Threshold at 1mV – Similar to RMT, the motor threshold at 1mV also known as MT1 (Fig.6) is defined as the minimum stimulation intensity necessary to evoke MEPs with a peak to peak amplitude of ≥ 1mV in 5 out of 10 consecutive single pulse deliveries. (Fig.6)

3. Cortical Silent Period – TMS pulse applied to the motor cortex can have both excitatory and inhibitory effects depending on the state of the muscle. At resting state as in case of RMT and MT1, the single pulses delivered activate the muscle giving rise to MEP. On the other hand, stimulating the motor hotspot while the target muscle is voluntarily contracted has an inhibitory effect on the said muscle's activity. Due to ongoing contraction in the muscle, TMS pulse results in a large MEP (usually above 1mV) followed by suppression of background EMG activity which may last up to hundreds of milliseconds (Fuhr et al., 1991). CSP(Fig.7) involves voluntary contraction of the FDI muscle which is then interrupted by suprathreshold stimulation for 10 consecutive pulses separated by 5seconds. Measurement of the silent period is from the end of the induced MEP to the beginning of EMG background activity and was done manually for all 416 subjects. Evidence suggests that the first 50ms of the CSP is contributed by spinal inhibition while the rest of it is because of cortical inhibition (Inghilleri et al., 1993; Chen et al., 1999). It has been observed that a GABA uptake inhibitor (Tiagabine) prolongs CSP duration (Werhahnet al., 1999). Multiple studies have also implicated $GABA_B$ receptor-mediated

inhibitory neurotransmission on CSP duration. (Nakamura et al., 1997; Saisanen et al., 2008)

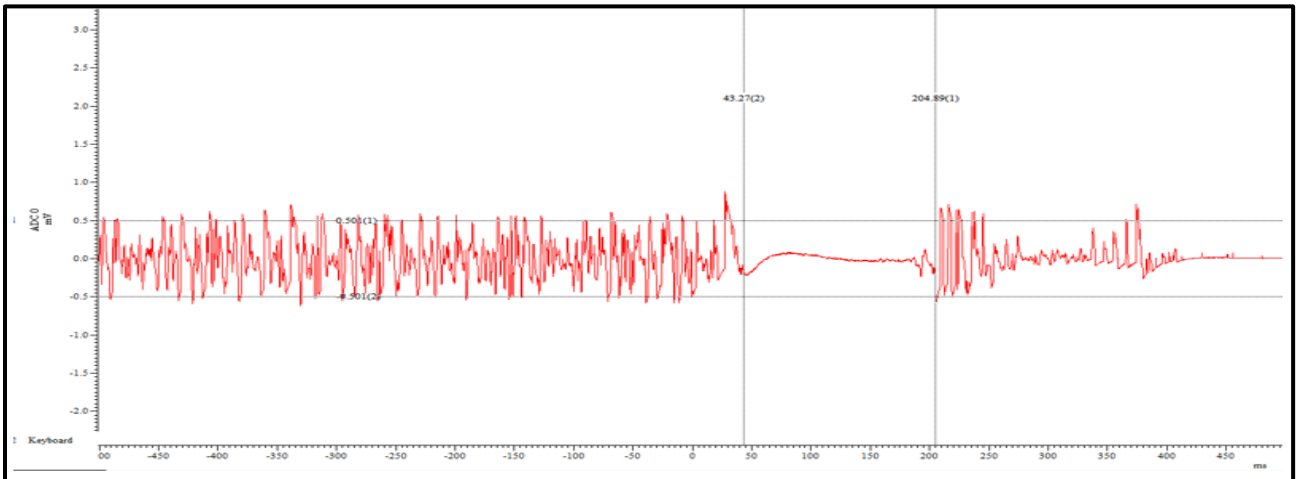


Fig.7 – CSP measurement in Signal Software. Trigger is given at time t=0. MEP is followed by refractory period. Vertical cursors mark CSP duration.

**Paired Pulse Paradigms –**

Paired-pulse stimulation is defined as the application of two sequential stimuli of different intensities separated by a predefined inter-stimulus interval (ISI). Depending on the ISI and the stimulation intensity, the effect can be excitatory (facilitation) or inhibitory (Kujirai et al., 1993; Ilic et al., 2002). The first pulse in all the paradigms is called conditioning stimulus (CS) and the subsequent pulse is known as test stimulus (TS). Studies have demonstrated that a subthreshold (below RMT) CS failed to give rise to MEP or any other response in cervical epidural electrode resulting in the conclusion that paired-pulse stimulation could be a result of motor cortical synaptic inhibition (Di Lazzaro et al., 2003). Inhibitory stimuli also did not seem to have any effect on spinal reflexes (Kujirai et al., 1993), which makes inhibitory paired-pulse stimulation a way to test corticocortical circuits specifically.

1. Short Interval intraCortical Inhibition (SICI) – In SICI (Fig.8), the CS is subthreshold at 80%RMT and TS is suprathreshold at MT1. The ISI is 3ms between the two stimuli. Resultant MEP has a small peak to peak amplitude as a result of inhibition. Currently, SICI is believed to be the product of axon refractoriness and ionotropic $GABA_A$ receptor-mediated inhibition (Ziemann et al., 1996). The MEP for SICI is measured between 10-70ms in Signal software. The %Inhibition caused by SICI is calculated as

(MEP after single pulse MT1-MEP after TS of SICI) *100/ (MEP after single pulse MT1)

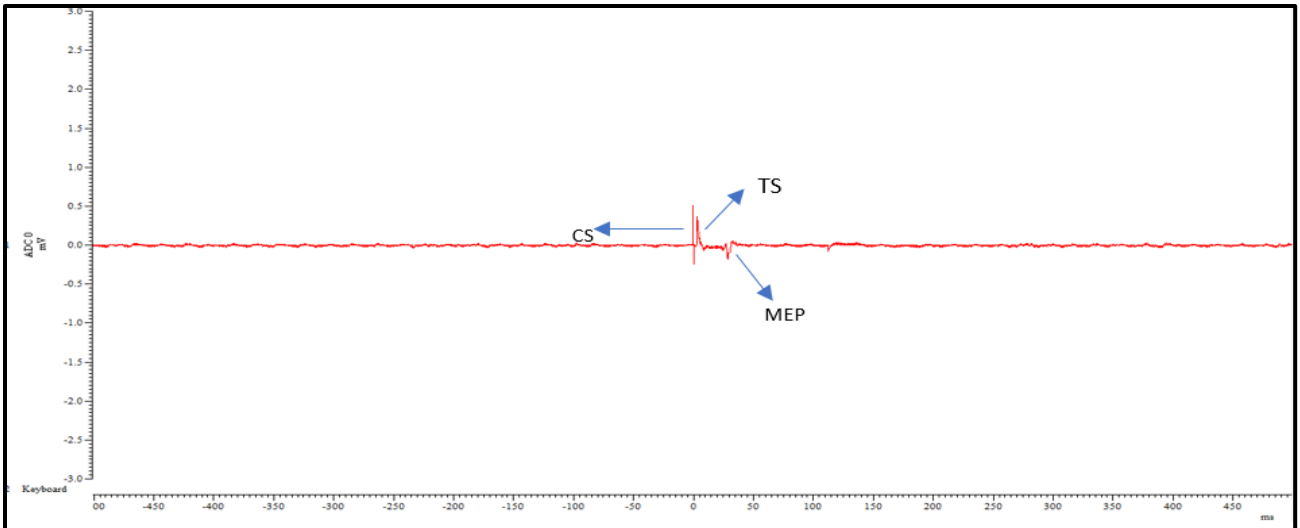


Fig.8 – Short Interval intracortical Inhibition (SICI). CS is subthreshold at 80%RMT and TS is suprathreshold at MT1. ISI is 3ms

2. Long Interval intracortical Inhibition (LICI) – In LICI, the CS and TS are both suprathreshold at MT1 separated by ISI of 100ms (Fig.9). LICI is believed to be a result of postsynaptic $GABA_B$ receptor-mediated activity as well as a function of cortical silent period (Valls-Sole et al., 1992). In general, the value of MEP after TS of LICI is smaller than that of SICI. The MEP for LICI is measured between 120-170ms in Signal Software. The %Inhibition caused by LICI is calculated as

(MEP after single pulse MT1-MEP after TS of LICI) *100/ (MEP after single pulse MT1)

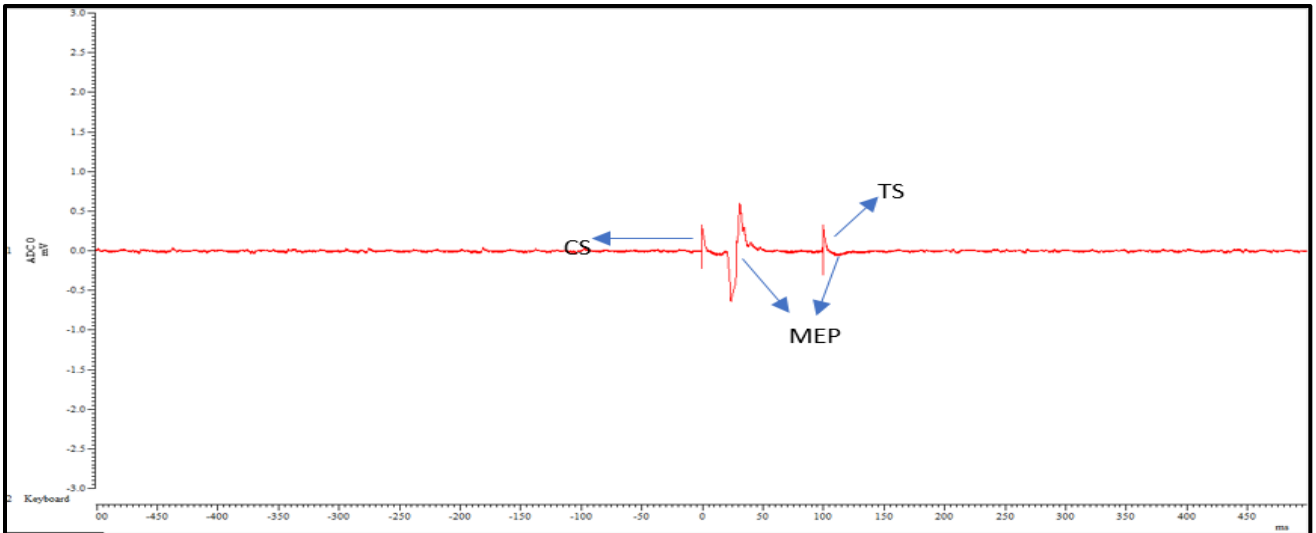


Fig.9 – Long Interval intracortical Inhibition (LICI). Both CS and TS are suprathreshold at MT1. ISI is 100ms.

The protocol set in MagPro R30 with MagOption contained 10 SICIs, 10 LICIs and 10MT1s interspersed with each other and the sequence of which was randomized to reduce the possibility of habituation.

**Limitations** –

1. The entire investigation requires for the coil to be held at a constant angle at the motor hotspot which may not always happen due to the human factor. The location of the hotspot is marked on the head in order to reduce this error.
2. The localization of M1 hotspot can prove to be a time-consuming process in case the motor hotspot is pinpointed and which can cause muscle fatigue in both the subject as well as the investigator. The subject's muscle fatigue can be remedied by asking them to voluntarily contract and relax hand muscles.
3. Determination of RMT or even M1 can prove to be tricky in which case Active motor threshold (AMT) (with protocol same as CSP) can be used to determine both since AMT is 80-90% of RMT.
4. The assessments included in the study had major paradigms CSP, SICI, LICI and MT1 common which have been used to do the transdiagnostic analysis. Certain paired-pulse paradigms involving facilitation was used in very few assessments and hence have not been studied across disorders.

**Statistical Analysis** – All of the processed data was compiled in Microsoft Excel and statistical analysis was run using Rcommander-EZR package in R_studio, Boston, MA, which is an open-source statistical analysis software.

Categorical variables were analyzed with Chi-squared Test and the rest of the continuous variables were analyzed using One-way Analysis of Variance (ANOVA) with the application of a post-hoc Tukey criterion (Significance at $\alpha \leq 0.05$). Analysis of Covariance (ANCOVA) was done for one variable to adjust for contributing variables other than disorders. Pearson's correlation coefficient ($\alpha \leq 0.05$ for significance) was used to test the association between two continuous variables.

## RESULTS

Sociodemographic as well as TMS variables such as Gender, years of education and age were analyzed for 6 groups i.e. Healthy Controls (HC), Depression (D), Schizophrenia (S), BPAD Mania (Mania), High-Risk BPAD Mania (HR) and Obsessive-Compulsive Disorder (OCD).

### I. Groups match for age and gender

The six groups are not significantly different ($\alpha>0.05$) with respect to Age (Fig. 10a) and Gender while they differ in terms of years of education (Table 1). It can be seen from the percentages in Table 1, that healthy control recruitment since it is done on the NIMHANS campus involves a more educated population than the rest of the groups. Psychiatric disorders have onset during adolescence and early adulthood (Lijster et al., 2017; Dagani et al., 2017) which might disrupt their education. The difference in educational status may also be secondary to a bias in sampling, recruited from a public-funded hospital.

Since, the samples were obtained as part of different studies carried out through a time period of eight years, individual subject matching between groups could not be done. Further, there were differences in sample size between the groups. Nevertheless, the groups are similar in mean age and gender proportions which makes it possible to compare other parameters of the studies.

| Group | Age (μ±σ) | Gender | | Years of Education | | | |
|---|---|---|---|---|---|---|---|
| | Years | M | F | <5 | 5 to 12 | 13 to 15 | >15 |
| HC(n=121) | 30.67±7.6 | 78 (60.2%) | 51 (39.8%) | 7 (6.67%) | 15 (14.28%) | 45 (42.85%) | 36 (34.29%) |
| D (n=91) | 33.18±8.67 | 58 (58%) | 42 (42%) | 6 (6.59%) | 39 (42.86%) | 25 (27.47%) | 21 (23.08%) |
| S (n=61) | 31.93±8.48 | 46(51.11%) | 44 48.89%) | 1 (0.02%) | 37 (60.60%) | 19 (31.15%) | 4((6.56%) |
| Mania (n=28) | 33.36±10.53 | 18(64.29%) | 10(35.71%) | 6 (21.42%) | 19 (67.86%) | 2 (8.14%) | 1 (3.57%) |
| HR (n=30) | 29.75±6.39 | 23(76.67%) | 7(23.33%) | 1 (3.33%) | 21 (70%) | 7 (23.33%) | 1 (3.33%) |
| OCD (n=33) | 29.28±9.19 | 29(76.32%) | 9(23.67%) | 3 (9.09%) | 10 (30.30%) | 14(46.67%) | 6 (20%) |
| Test | ANOVA | χ squared | | χ squared | | | |
| p-value | 0.079 | 0.051 | | **<0.001** | | | |

**Table1** – ANOVA and χ-squared test results for Age, gender and years of education matching. Mean and standard deviation calculated from raw data.

## II. Motor Thresholds

The values in Table 2 and Fig. 10b, 10c, 10d represent machine output percentage for all subjects. RMT and MT1 values for all 416 subjects are highly correlated (r=0.861, p<0.0001, 95%CI=0.834-0.885; Fig.10b). RMT (r=0.108, p=0.421; Fig.10c X-axis) shows no significant correlation with age (Fig. 10c, Y-axis) whereas MT1 (r=0.156, p=0.00307; Fig.10d, X-axis) does. ANCOVA of MT1 (Fig.11a, X-axis) with disorder groups (individual lines) adjusting for age (Fig.11a, Y-axis) gave p=0.006. The MT1 value increases with age within groups (Fig.11a). There is no significant difference between RMT (p=0.739) and MT1 (p=0.745) values between genders. The data can be compared between groups. Mean RMT and MT1 are lowest for OCD and highest for Mania group (Fig.11 b & c). ANOVA followed by post-hoc Tukey test showed that RMT of OCD group is significantly lower than Schizophrenia, Mania, Healthy Control (p<0.001 for all) and Depression (p<0.05) while is comparable to High-Risk BPAD group. Pairwise comparison of RMT between Depression and other groups shows that mean RMT of Depression is significantly lower (p<0.05) than Mania and Schizophrenia. Pairwise comparison between Depression, HC and HR yielded no significant difference (p>0.05) and Schizophrenia and Mania groups are also comparable to one another (Table 2). OCD group shows low variability (narrow standard deviation/SD) whereas Mania and Schizophrenia groups have high variability (wider SD) both in RMT as well as MT1 outputs (Fig. 11 b & c)

| Group | RMT (μ±σ) | MT1 (μ±σ) |
|---|---|---|
| HC (n=130) | 35.25±6.38 | 47.15±9.9 |
| D (N=91) | 33.92±6.35 | 45.82±8.92 |
| S (n=88) | 37.02±9.53 | 49.28±12.16 |
| Mania (n=28) | 38.75±9.27 | 53.54±12.30 |
| HR (30) | 34±5.38 | 41.83±6.77 |
| OCD (38) | 29.35±4.0 | 37.43±5.69 |
| Test | ANOVA | ANOVA |
| p-value | **<0.001** | **<0.001** |

**Table 2** – Result of ANOVA for Motor threshold values for all six groups.

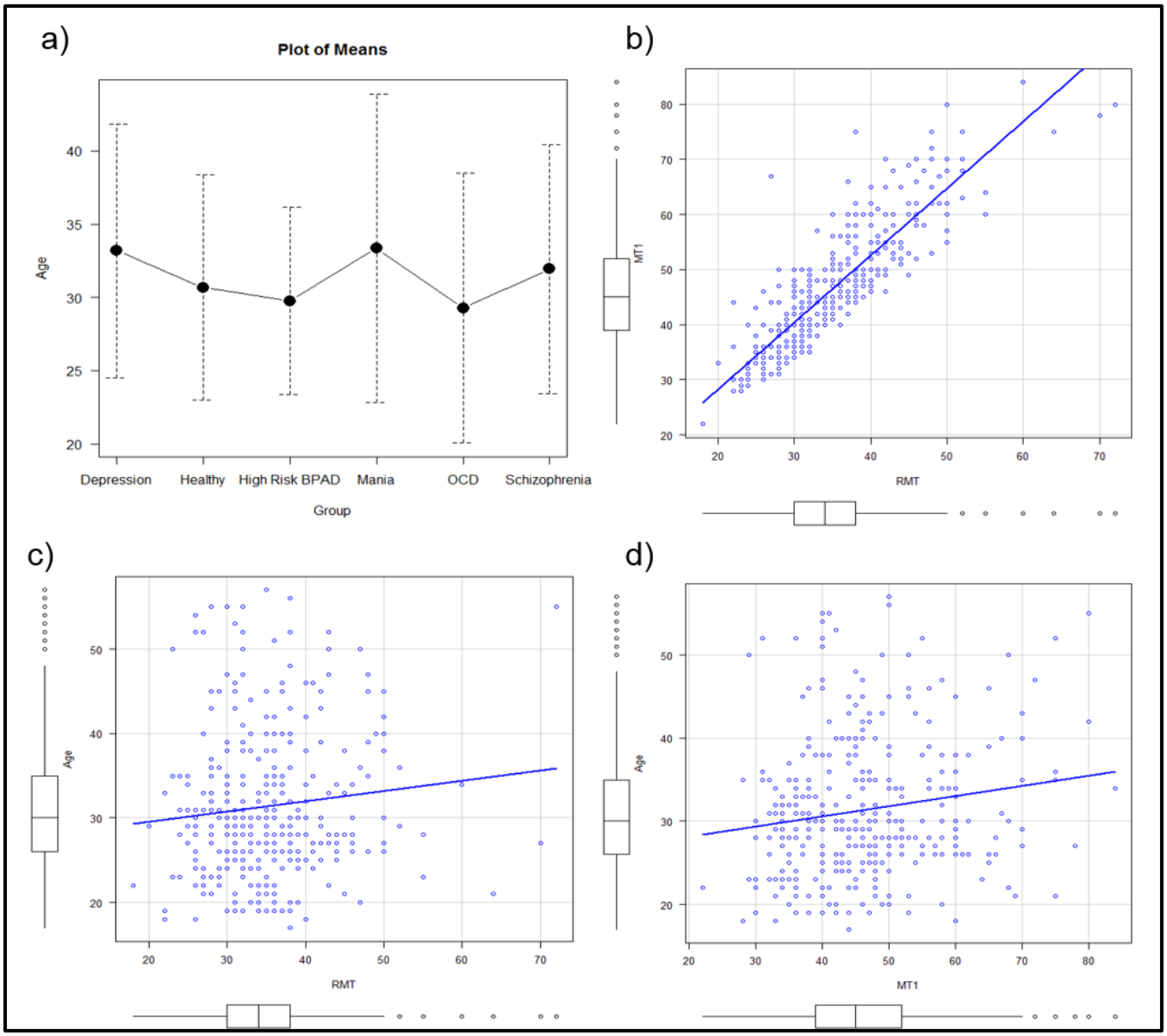


**Fig.10** – Sociodemographic and TMS Plots. a) Line plot of mean age (in years) of groups, b) Scatter plot with the correlation between RMT(x-axis) between MT1(y-axis), c) Scatter plot with the correlation between RMT(x-axis) and Age (y-axis) as well as d) MT1(x-axis) with age.

Similar to RMT, post-hoc Tukey shows that mean MT1 values of the OCD group are significantly lower ($p<0.005$) from all groups other than High-Risk BPAD. Mean MT1 values of Mania are significantly higher ($p<0.05$) for all groups other than Schizophrenia. (Table 2; Fig.11 b & c)

Low values of RMT are indicative of higher motor cortical excitability as RMT is the

minimum machine output required to activate the muscle 50% of the times. This also shows Mania and Schizophrenia motor cortex to have lower excitability.

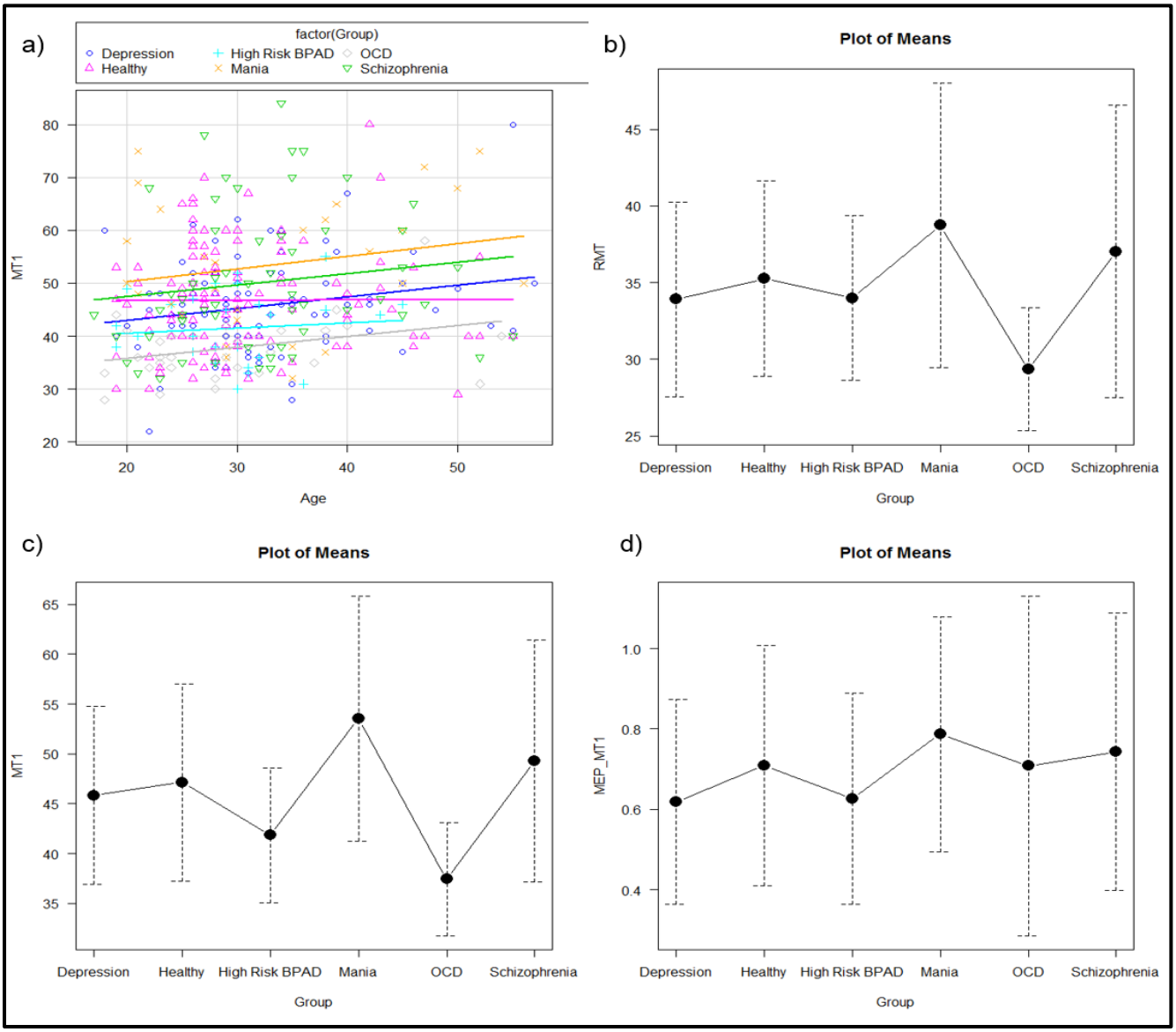


**Fig.11** – Plot of motor threshold values for groups. a) ANCOVA of MT1 with groups adjusting for age, Line plots of mean b) RMT value of groups, c) MT1 value of groups, d) MT1 MEP of groups.

### III. MEPs of SICI & LICI parameters differ significantly across groups.

MEPs, recorded by running a preset randomized sequence in MagPro R30 with MagOption and processed by Signal software, are shown in Table3.

No significant correlation observed between MT1 MEP (r=-0.0878, p>0.05), SICI MEP (r=0.0343, p>0.1) and LICI MEP (r=-0.0925, p>0.05) with age. They also do not show any

significant difference between genders (all p>0.1). The data can be compared between groups.

The MT1 MEPs show a significant difference between groups in ANOVA, only pairwise comparison of Schizophrenia-Depression shows p<0.05 while the other combinations of groups are comparable to one another (Table 3, Fig. 11d).

Groups differ significantly when SICI MEPs are compared with ANOVA (Table 3; Fig 12b). Pairwise comparison of Mania with HC and HR shows p<0.05. Rest of the group pairs are comparable to one another. There is no difference shown by groups in % inhibition caused by SICI (Table 3, Fig.12c). Even though there is significance shown by MT1 MEP and SICI MEP analysis, inhibition when taken into account is happening to a similar extent in the patient population as well as the healthy controls. Inhibition resulting from SICI happens to similar extend in these psychiatric disorders as well as healthy subjects. MT1 MEPs (Fig. 12a, Y-axis) and SICI MEPs (Fig. 12a, X-axis) of all 416 subjects are correlated (Pearson's r=0.603, 95%CI=0.536-0.663, p-value<0.001; Fig.12a). This might be indicative of similar if not related underlying mechanisms for the two parameters.

| Group | MT1 MEP | SICI MEP | % inhibition SICI | LICI MEP | % inhibition LICI |
|---|---|---|---|---|---|
| | mV | mV | | mV | |
| HC (n=130) | 0.71±0.3 | 0.49±0.29 | 14.86±117.04 | 0.30±0.30 | 52.5±43.12 |
| D (n=99) | 0.62±0.25 | 0.52±0.30 | 14.77±38.74 | 0.36±0.21 | 32.09±52.83 |
| S (n=89) | 0.75±0.35 | 0.58±0.32 | 10.95±54.45 | 0.26±0.24 | 60.56±34.53 |
| Mania (n=28) | 0.79±0.29 | 0.69±0.30 | 6.65±42.73 | 0.15±0.14 | 77.78±20.55 |
| HR (n=30) | 0.63±0.26 | 0.41±0.28 | 29.51±39.5 | 0.18±0.14 | 65.22±28.51 |
| OCD (n=38) | 0.71±0.42 | 0.56±0.39 | 12.30±49.04 | 0.73±0.37 | -97.38±338.57 |
| Test | ANOVA | ANOVA | ANOVA | ANOVA | ANOVA |
| p-value | **0.003** | **0.002** | 0.106 | **<0.001** | **<0.001** |

**Table3** – ANOVA analysis of MT1 MEP, SICI MEP, % Inhibition SICI, LICI MEP and % Inhibition LICI. Mean and standard deviation calculated.

% Inhibition SICI (LICI) is calculated using the formula

(MT1 MEP-SICI (LICI) MEP) *100/ (MT1 MEP). This also explains the inverse trend (high SICI (LICI) hence low % Inhibition SICI (LICI)) in Fig. 12 b & c and Fig. 13 a & b.

Groups show high inter-individual variability (wide SD) in MT1 MEP (Fig. 11d), SICI

MEP (Fig. 12b) and % Inhibition SICI (Fig. 12c).

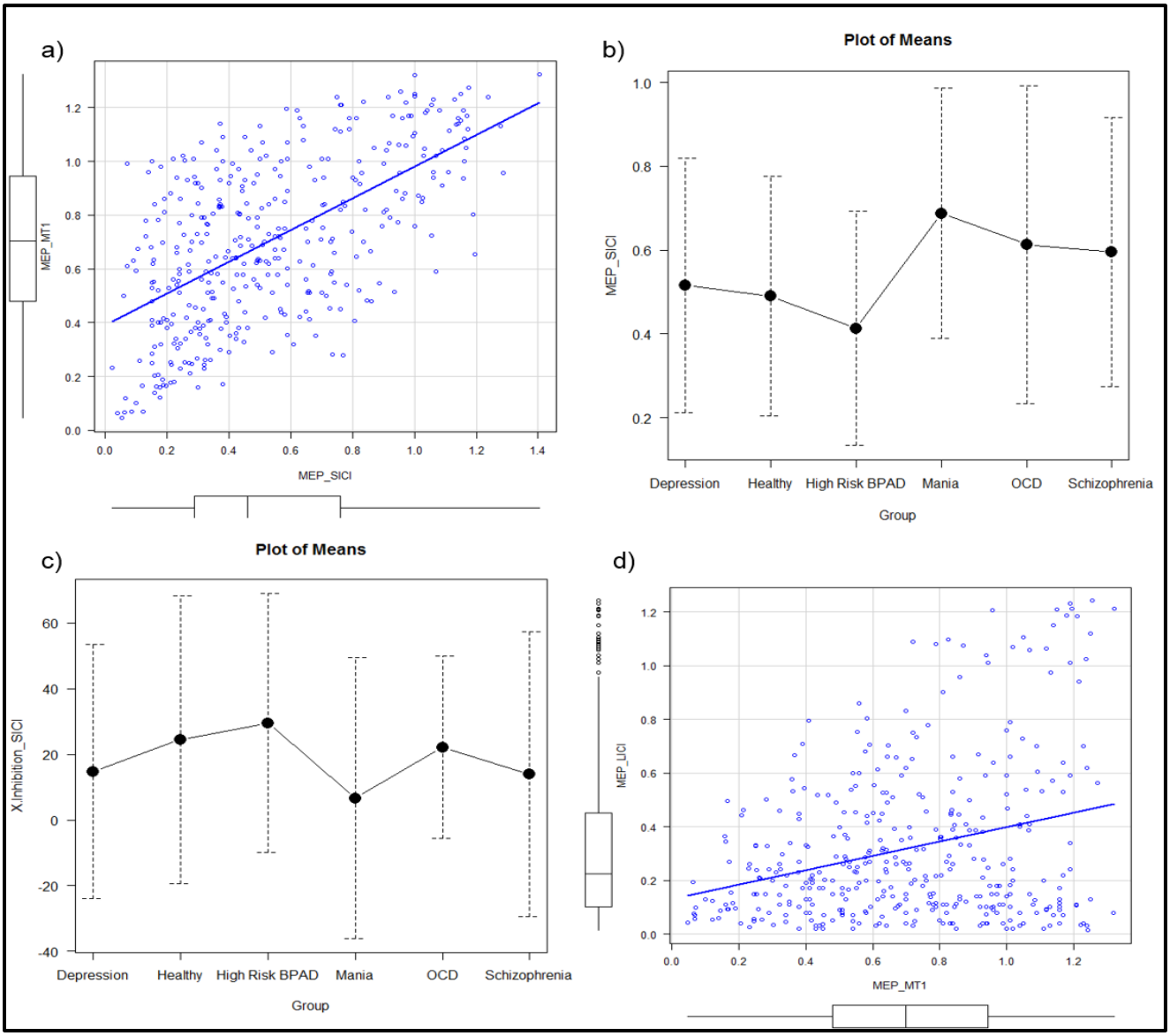


**Fig.12** – Motor Evoked Potentials across groups. a) Pearson's correlation between SICI MEP and MT1 MEP, b) Line plot of mean SICI MEP in each group, c) Line plot of %Inhibition caused by SICI in each group, d) Scatter Plot for correlation between LICI MEP and MT1 MEP

LICI MEPs show OCD group to be significantly higher than all the other groups and consequently %Inhibition by LICI is also lower in OCD as compared to other groups (Table 3; Fig.13 a & b). All the rest of the groups as comparable in both aspects. OCD and HC groups show high variability (wide SD) in LICI MEPs as well as %inhibition. LICI MEPs and MT1 MEPs show low correlation ($r=0.235$, $p<0.0001$; Fig.12d). OCD shows high inter-individual variability (wide SD) in LICI MEP and % Inhibition LICI (also seen in

Depression). Mania shows narrow SD as well as high % Inhibition LICI values. High values of LICI MEP are indicative of the conditioning stimulus not being effective in reducing the effect generated by the test stimulus leading to lowered inhibition.

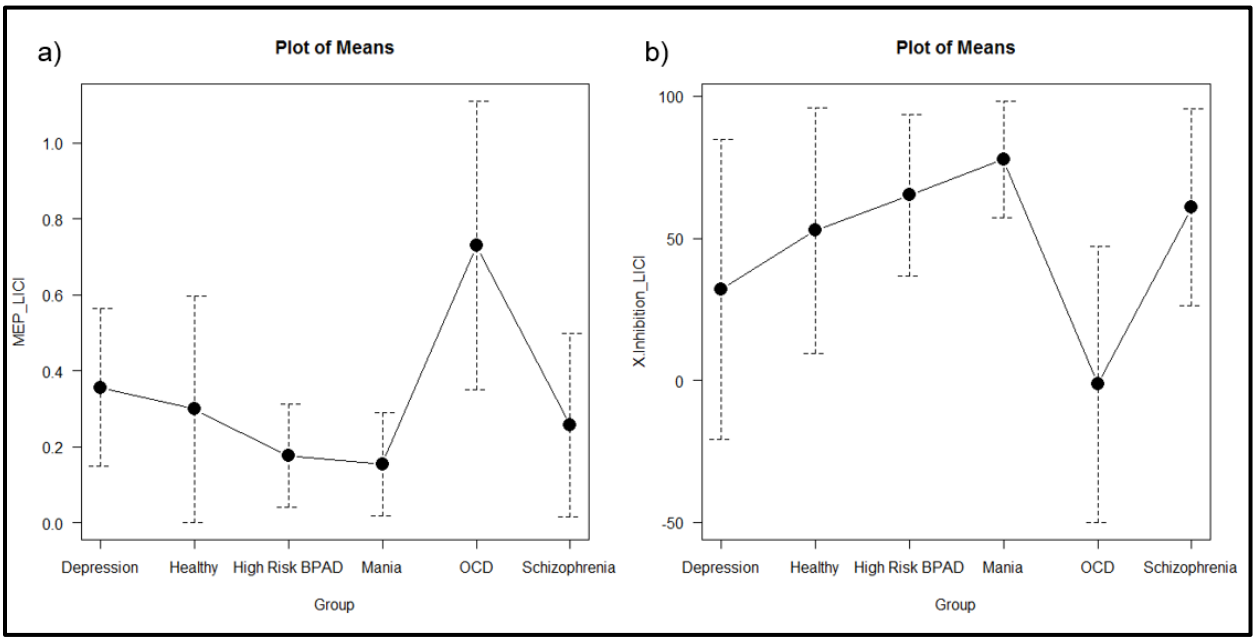


**Fig.13** – Long Intracortical Inhibition across groups. a) Line plot of Mean LICI MEP in each group, b) Line plot of mean %Inhibition caused by LICI in each group. The pattern of LICI MEP values and %Inhibition caused by LICI exhibited by groups can be seen.

### IV. Cortical Silent Period (CSP) is lowest for OCD and high for BPAD Mania

CSP files of all 416 subjects were processed and CSP was measured in Signal Software manually. CSP values show no correlation with age ($r=-0.0137$, $p=0.7960$). No significant difference observed between CSP measures of the two genders ($p=0.617$).

CSP values of the OCD group is significantly lower ($p<0.05$) from all other groups (Table 4; Fig.14a). CSP in Depression was significantly lower than Healthy Control ($p<0.05$). Schizophrenia shows a significant difference from the HR group ($p<0.05$). OCD and Depression have lowered silent period indicating deficits in inhibition in the disorders.

CSP shows no correlation with %inhibition by SICI ($r=0.0582$, $p=0.25$) and low correlation with %inhibition by LICI ($r=0.202$, $p<0.0001$, Fig.14b). This indicates LICI has some similar mechanisms to CSP but there might be other factors involved during inhibition. Groups show high inter-individual variability as seen from wide standard deviations(Fig

14a).

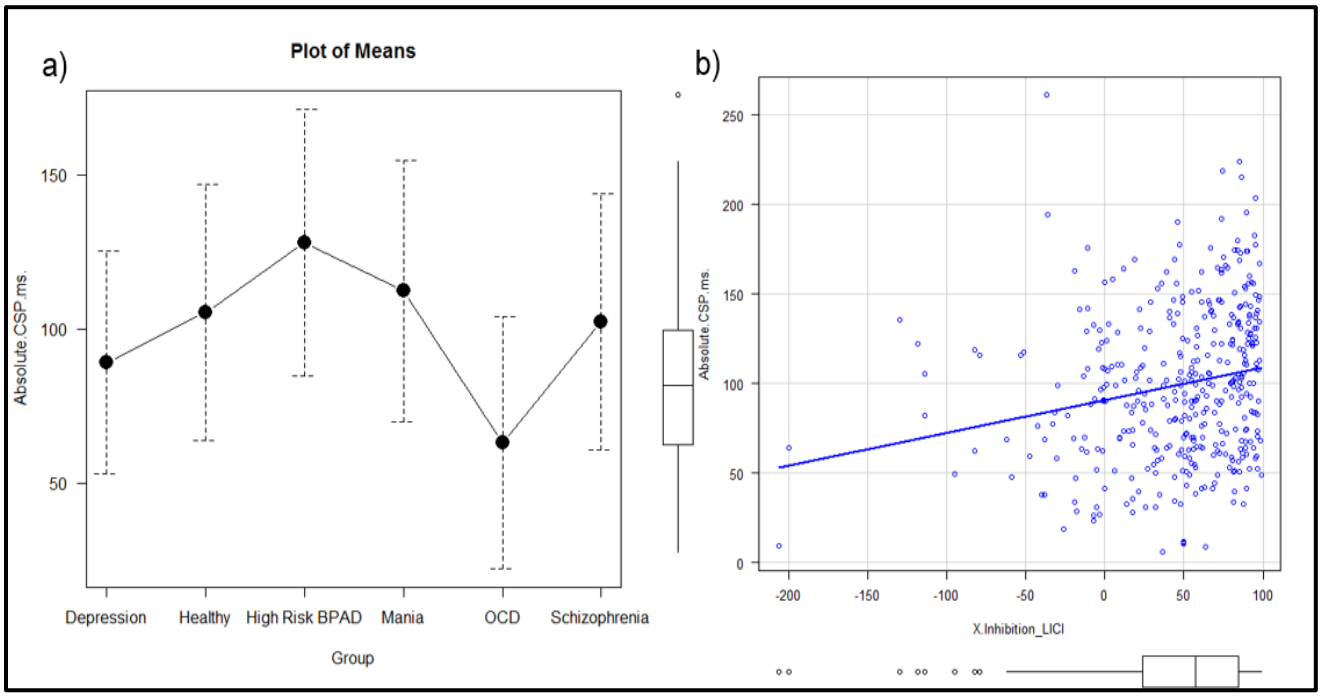


**Fig.14** – Silent Period. a) CSP values across groups, b) Scatter plot with the correlation between CSP and %inhibition due to LICI

| Group | CSP |
|---|---|
| | mS |
| HC (n=130) | 105.37±41.68 |
| D (n=100) | 89.05±36.18 |
| S (n=90) | 102.36±41.51 |
| Mania (n=28) | 112.42±42.46 |
| HR (n=30) | 128.04±43.26 |
| OCD (n=38) | 63.14±40.85 |
| Test | ANOVA |
| p Value | **<0.001** |

**Table4** – Result of ANOVA for Cortical Silent Period between groups.

## DISCUSSION

The current study aimed to evaluate the difference in various motor cortical excitability measures as measured by TMS across subjects with various psychiatric disorders in comparison with healthy controls. By collating and analyzing the data, we are able to see certain similarities and differences in individual excitability measures between the different groups. Age and gender matching of groups allows us to compare the groups. The baseline analysis showed that age or gender did not have any significant correlation with most of the variables studied. Hence the presence of differences which might aid in understanding and speculating the underlying neurobiological differences between disorders is striking. The present study is not only comparing individual disorders with healthy subjects but also looking at differences between them and the trends in TMS parameters they might bring up.

Primary excitability parameters i.e. RMT and MT1 values are low in OCD followed by Depression indicating higher cortical excitability in these disorders. A few previous studies on OCD, MDD and Schizophrenia have reported no significant difference between these excitability measurements between subjects and healthy volunteers (Radhu et al., 2013; Kaskie and Ferrarelli, 2018). Schizophrenia and BPAD Mania have higher motor threshold as compared to patients with MDD but not with healthy controls. The highly significant correlation observed between RMT and MT1 thresholds indicates the utilization of similar excitatory mechanisms (involving NMDA and voltage-gated $NA^{+}$ and $Ca^{2+}$ channels) and motor pathways for these single pulse paradigms (Chen et al., 1997; Ziemann et al., 1996).

Per cent inhibition caused by SICI is not significantly different between groups (Table3; Fig.12c) indicating no significant $GABA_A$ impairment in subjects. Although individual studies (Radhu et al., 2013; Kaskie and Ferrarelli, 2018) have reported significant reduction in SICI in certain disorders as compared to healthy controls, the current data do not support these findings. Several studies have reported significant reduction in SICI in patients suffering from Schizophrenia, MDD and OCD as compared to healthy controls. A meta-analytic study found reduced SICI in patients with Schizophrenia, MDD and OCD (Radhu et al., 2013). Also, previous studies have not been entirely consistent with the

findings (Steele et al., 2000; Richter et al., 2012; Mehta et al., 2014) These contradictory findings may be secondary to difference in sample characteristics such as age, gender, duration of illness, ongoing treatment, severity of symptoms etc. Further, the possibility of Type-I statistical error cannot be ruled out. There also appears a significant correlation between MT1 MEP amplitude and SICI MEP amplitude (Fig.12a) hinting at the possibility of the presence of other mechanisms along with $GABA_A$ receptor-mediated inhibition. Although there have been some correlations with imaging studies, a clear causal connection is unknown (Robert Chen, 2004).

The non-significant difference in SICI inhibition between healthy controls and patients might also be due to the higher variability (standard deviation) observed in results from healthy control. Although there is inter-individual variability observed in TMS assessments, the TMS parameters not only depend on the absence of a diagnosed disorder but also physical and environmental factors which can be remedied for while conducting the assessment. Hence, there is a need for studies to standardize the timing and conditions of cortical excitability assessments. As the samples were obtained as part of different studies, such standardization was not possible in the current data.

Motor evoked potential measured for LICI was highest in OCD (Fig.13a) which is in agreement with low per cent inhibition caused by LICI (Fig.13b). OCD patients also show low Cortical Silent Period when compared with healthy controls as well as other disorders (Fig.14a) which strengthens the speculation that reduced $GABA_B$ receptor-mediated inhibition is more prominent in OCD patients than others. This is in line with previous meta-analytic findings and individual studies (Radhu et al., 2013; Kaskie and Ferrarelli, 2018). Animal studies focusing on OCD (more specifically development of rodent models for OCD and their validity) have implicated cortico-striatal-thalamic-cortico (CSTC) circuit in the neurobiology of OCD where striatum activates thalamus via a direct pathway and inhibits thalamus via GABAergic indirect pathway. Studies have suggested the involvement of multiple neurotransmitters in the neurobiology of OCD like 5-HT, myoinositol, glutamate, GABA and Dopamine (Kroff and Harvey, 2006). GABAB Receptor 1 gene polymorphs have also been indicated to be positively correlated with the development of OCD in mice (Zai et al., 2005). Use of TMS on rodents has been to test the safety and effects of treatment paradigms. Similar animal studies along with human

would also be useful in understanding the circuits involved in various paradigms. Induced stress has been found to reduce GABA levels in rat hippocampus (Harvey et al., 2004). MDD patients display lowered CSP and lower per cent inhibition by LICI than the healthy control while Schizophrenia patients do not which is in agreement with individual studies done with the aforementioned disorders (Kaskie and Ferrarelli, 2018). Mood disorders that primarily involve anxiety or stress have studied in various rodent models have shown GABAB dysfunction (Brambilla et al., 2003).

OCD and MDD are both termed as neurotic disorders (ICD-10). Neurosis is a class of psychiatric disorders involving negative emotions including anxiety which obstructs functioning. Results from analysis reflect a deficiency in $GABA_B$ receptor-mediated inhibition in neurotic disorders mentioned above. Additionally, drugs acting on GABA receptors such as benzodiazepines are known to decrease anxiety, further supporting the hypothesis that decreased GABA functioning may cause anxiogenic effects in these disorders. These disorders were also associated with lower (though not significantly) RMT as compared to healthy volunteers suggesting increased excitability. Due to the absence of excitatory/facilitatory paired-pulse paradigm, the increased motor cortical excitability cannot be commented upon.

Although the study does not show a significant difference in RMT, MT1, LICI inhibition and CSP between BPAD Mania and other groups clearly, we see that BPAD mania patients display higher RMT, MT1 values and also higher LICI inhibition and CSP. They also show low inhibition by SICI. We speculate that BPAD Mania patients might have heightened $GABA_B$ and reduced $GABA_A$ receptor-mediated inhibition. Albeit a low number of data points in this group, the group shows low variability in excitability and inhibitory parameters. Although none of these analyses reached statistical significance, similar trends across measures suggest that there is merit in testing this hypothesis in larger samples after controlling for necessary measures.

Similar to the aforementioned observations, studies have shown combinations of abnormalities in GABAergic inhibition and intracortical facilitation might be involved in the pathophysiology of OCD, MDD and Schizophrenia (Radhu et al., 2013). We can speculate something similar happening in BPAD Mania as there might exist a pattern of cortical inhibitory and excitatory parameter incongruency that is particular to a psychiatric

disorder. Although in case of OCD, studies show inhibitory deficiency as well as enhanced facilitation (Greenberg et al. 1998, 2000; Richter et al., 2012), there are reports in MDD (Levinson et al., 2010) and Schizophrenia patients (Daskalakis et al., 2008a; Liu et al., 2009) where the type of disorder (treatment resistance) or medication might be playing a bigger role in modulating cortical excitability than we realize.

Altogether this study hints at the presence of an existing pattern in the combinations of GABAergic inhibition and Glutamatergic facilitation by single as well as paired-pulse TMS paradigms. The mechanisms by which the paired-pulse paradigms of TMS operate in the motor cortex and how they affect the surrounding areas via transsynaptic mechanisms are not yet completely understood. Studies utilizing a combination of paired-pulse paradigms help not only to understand specific patterns of inhibition and facilitation existing in the healthy and diseased brain but also to understand mechanisms underlying the paradigms themselves. It is possible that both, single and paired-pulse, might be affecting each other which would also give knowledge about the time period of their action and hence the underlying neurobiology.

To summarize, the current study suggests a $GABA_B$ mediated dysfunction in OCD and to an extent in MDD. Although there is a hint towards GABA dysfunction in various other disorders including bipolar disorders, these have to be confirmed in studies with larger sample sizes. As facilitatory paradigms were not studied adequately, glutamatergic functioning cannot be commented upon based on the current study. Nevertheless, these are interesting prospects for future studies as other lines of evidence suggest a glutamatergic dysfunction in some of these disorders such as OCD, MDD and schizophrenia (Radhu et al., 2013; Kaskie and Ferrarelli, 2018).

The major limitation of the study is that the sample was recruited as a part of different studies and hence the selection criteria were not uniform and the samples were not matched on important confounders. Many of the patients were on some medications, which might have confounded the results (Levinson et al., 2010; Daskalakis et al., 2008a; Liu et al., 2009). Notwithstanding this, the strength of the study was that it was first of its kind to compare the excitability measures using the same instruments and similar protocols across different psychiatric disorders. The use of the same instruments makes the data directly comparable as compared to meta-analytic studies as in meta-analyses,

the motor thresholds from individual studies can be from different instruments with varying sensitivities. The study throws up interesting findings which have to be compared in future studies with larger samples after matching for potential confounders.

TMS studies in augmentation with Electroencephalography, Magnetic Resonance Imaging and other imaging tools may also aid in this quest. This will also help us in developing more targeted pharmacological and non-pharmacological treatment paradigms for these conditions. Further, measuring these paradigms before and after various treatments such as repetitive TMS (rTMS), Theta Burst Stimulation (TBS) treatment and Transcranial Direct Current Stimulation (TdCS) treatment would help in understanding the mechanism of action of these treatments and help in refining the treatment modalities.

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