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Diagnostic value of combined transcranial magnetic stimulation (TMS) and electroencephalography (EEG) in epilepsy.

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DIAGNOSTIC VALUE OF COMBINED TMS AND EEG IN EPILEPSY

by

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This dissertation is submitted for the degree of

Doctor of Philosophy (PhD)

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Declaration

I hereby declare that except where specific reference is made to the work of others, the contents of this dissertation are original and have not been submitted in whole or in part for consideration for any other degree or qualification in this or any other university. This dissertation is my own work and contains nothing which is the outcome of work done in collaboration with others, except as specified in the text and acknowledgements.

This PhD is dedicated to the memory of my beloved father, **Santos Lázaro Andaluz**, for the love, care and devotion that he always provided for his family and for always being proud of us.

Dad, thank you for the countless sacrifices you made to help me on my journey, supporting me unreservedly and teaching me the importance of believing in myself. Without your courage, generosity, and love, my life would never have been the same.

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Abstract

Purpose: The main aim of the project is to estimate the value of combined TMS-EEG responses and EEG to increase the sensitivity and/or specificity of the routine EEG in the diagnosis of newly onset epilepsies. **Methods:** The project is a combined cross-sectional and longitudinal study involving 60 patients recruited from the First Seizure Clinic at Guy's and St Thomas Hospital NHS Foundation Trust who have had their first presumed epileptic seizure. All the participants had a sleep-deprived EEG (baseline EEG) followed by a combined TMS and EEG study (TMS-EEG). The EEG responses to TMS were visually analysed, looking for two different types of TMS-evoked responses or late responses: The delayed responses were assessed in the unprocessed EEG and the repetitive responses (RRs) after averaging the EEG signals synchronized with the TMS pulse. The late responses were compared between epileptic and non-epileptic patients, looking for responses associated with epilepsy. In patients where the baseline EEG was normal, the additional diagnostic value provided by TMS-EEG was estimated by their ability to predict the final diagnosis based on the clinical history and other tests. A quantitative analysis was performed to compare the power ratio in different frequency bands between epilepsy and no epilepsy cohorts and to select epilepsy-associated variables to generate a machine learning-based classification model for epilepsy prediction. **Results:** In patients with normal baseline EEG, abnormal TMS-EEG evoked responses (late responses) had no statistically significant association with the presence of epilepsy (Fisher’s exact test, p=0.063), but the late responses correctly classified as epilepsy the 36% of patients with a false-negative baseline EEG. The combined presence of late responses and interictal epileptiform discharges (IEDs) in TMS-EEG records has a higher sensitivity (74%) but lower specificity (85%) than baseline EEG alone. The grand average power-ratio

differences between epilepsy and no-epilepsy cohorts were not statistically significant. The epilepsy-associated variables selected for machine learning-based classification were predominantly in the alpha-theta and gamma frequency ranges when TMS activation was present and, in the beta-gamma range with Sham. The TMS support vector machine (SVM)-classifier's disease prediction over an independent cohort had a sensitivity of 83%. **Conclusions:** The TMS-EEG significantly increased the sensitivity of the baseline EEG and correctly classified as epilepsy approximately one-third of the patients with a false negative baseline EEG and a final clinical diagnosis of epilepsy. TMS stimulation modified the spectral and topographic properties of the epilepsy-associated variables used for disease detection with machine learning linear regression algorithms. The performance of the TMS SVM-classifier in the training cohort has a high sensitivity, high specificity and low misclassification rate. The TMS SVM-classifier performed better than the Sham as an epilepsy disease prediction model in an independent TMS-EEG cohort. The TMS SVM-classifier has a promising value for disease prediction in TMS-EEG datasets.

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CHAPTER 1. INTRODUCTION

C.1.1. Epilepsy

C.1.1.1. General introduction to epilepsy

Epilepsy is a brain disease characterised by a chronic predisposition to suffer from recurrent, unprovoked, uncontrollable seizures (Fisher et al., 2014), and it is still, these days, one of the most commonly encountered neurological conditions. This condition has multifactorial causes reflecting acquired and genetic factors. Epilepsy has an incidence of 50-74/100,000 per year and a lifetime prevalence of 5 -10/1,000 (Fiest et al., 2017, Beghi, 2020). The World Health Organization (WHO) estimates that 50 million people worldwide suffer from epilepsy (WHO, 2019).

The epilepsies are broadly classified according to the type of seizure in focal, generalised, combined generalised and focal and unknown and according to aetiology in structural, genetic, infectious, metabolic, immune and unknown. The International League Against Epilepsy (ILAE) proposed a multi-level classification approach. Once the seizure type (first level of diagnosis) and the epilepsy type (second level of diagnosis) have been established, the diagnosis of epilepsy syndrome (third level of diagnosis) can be pursued. The epilepsy syndrome diagnosis combines the seizure type, the imaging studies and the electrographic features, and it also considers the aetiology, age of onset, and neuropsychiatric comorbidities (Scheffer et al., 2017, Rosenow et al., 2020, Koutroumanidis et al., 2017).

In focal epilepsies, the seizures arise from a localised brain area, while generalised epilepsies show a widespread involvement of both hemispheres from the outset of the seizures. The most common type of generalised epilepsy probably has a genetic basis (genetic generalised epilepsy-GGE). In contrast, focal epilepsies more often arise from

an abnormal focal anatomic substrate such as hippocampal sclerosis or an area of cortical dysgenesis.

The traditional etiological division of epilepsies in idiopathic or symptomatic served in the past for clinical and didactical purposes. However, this is an oversimplified approach that does not contemplate the interaction between genetic and environmental factors. For instance, genetic predisposition influences external factors, and the external insults would more likely cause the disease in genetically predisposed individuals (Berkovic et al., 2006). For that reason, the ILAE stance is that a patient may be classified into more than one etiological group. These groups do not have a pre-established hierarchical ranking, and its importance would depend on the specific case.

Approximately 57-79% of the newly diagnosed epilepsies in adults attain one-year remission, and 58% five years remission (Lindsten et al., 2001, MacDonald et al., 2000). There are several factors that have an impact on the natural evolution and prognosis of epilepsies, such as the number of seizures, responsiveness to treatment, aetiology and comorbidities, but in general, early recognition and appropriate medical treatment appear to reduce the recurrence of seizures (Mohanraj and Brodie, 2013). An early response to therapy predicts a good prognosis, and patients that are seizure-free within one year of receiving appropriate therapy are also likely to attain a five-year remission (Lindsten et al., 2001). Therefore, a timely and accurate diagnosis is crucial to improving the quality of life in patients with epilepsy.

However, due to its heterogenicity, the diagnosis of epilepsy is challenging (Leach et al., 2005), partially due to the brief, transitory nature and diversity of the interictal clinical and electroencephalographic manifestations. The gold standard for diagnosis

is a detailed and accurate clinical history supported by electroencephalographic abnormities consistent with the working diagnosis of a particular epilepsy syndrome (Koutroumanidis et al., 2017). Therefore, the scalp electroencephalogram (EEG) is essential for both the diagnosis and classification of epilepsy. However, abnormal EEG findings are not seen in every patient with epilepsy, and it is not always easy to make a distinction between generalised and focal epilepsy just on the basis of the clinical presentation or the EEG results (Scheffer et al., 2017, Rosenow et al., 2020, Koutroumanidis et al., 2017).

C.1.1.2. Physio pathological mechanisms in epilepsy

Seizures are the result of cerebral cortical and/or subcortical dysfunction leading to a series of events that generate hyperexcitability and increased neuronal synchronisation in a previously normal neuronal network. This has been suggested by in vivo animals' models of genetic and acquired epilepsies in which alterations in local cortical excitability and dysfunctional networks have been involved in the pathological mechanisms underlying epileptogenesis. Generalised and focal epilepsies have different pathophysiologic mechanisms.

Generalised genetic epilepsies

There are five potential theories regarding mechanisms of generalised epilepsy

1) Penfield and Jasper's centrencephalic theory suggests a deep midbrain pacemaker is responsible for triggering and maintaining synchronic bilateral discharges (Penfield, 1954).
2) Corticoreticular theory is linked to the thalamocortical mechanisms of spindles' generation and postulates that there is diffuse cortical hyperexcitability and a cortical overreaction to the physiological thalamic afferent inputs resulting in

generalised spike and wave discharges. The thalamus is secondarily involved in an oscillatory reverberating cortico-thalamic circuit (Gloor, 1968).

3) Cortical theory states that the primary abnormality in generalised epilepsy is at the cortical level, and the thalamus secondarily participates through normal physiologic thalamocortical interaction (Lüders et al., 1984, Niedermeyer, 1972, Bancaud et al., 1974).
4) Thalamic clock theory is a reformulation of the centrencephalic theory in which the reticular thalamic nucleus contains the pacemaker cells for a proposed thalamic clock. This thalamic clock is constituted by the recruitment of abnormal rhythmic oscillations in the intra-thalamic network, and it will determine the cortical rhythms. The thalamocortical relay cell (phase lock with spike and wave discharges) seems to precede neocortical cell firing by a few milliseconds, further supporting the thalamus as a generator of the cortical discharges (Buzsaki, 1991).
5) Cortical focus theory proposes that the spike-wave discharges have a focal onset in the cortex and are generalised through a rapid cortical propagation. In the first instance, the cortex drives the thalamic rhythm; after that, the cortex and thalamus drive each other amplifying and maintaining the discharges through corticothalamic loops (Meeren et al., 2005, Meeren et al., 2002).

The corticoreticular theory, in which both cortex and thalamus contribute to seizure generation, seems to be the most widely accepted. Gloor et al. proposed a thalamocortical mechanism explaining GGE in which thalamic inputs exerted over a pre-existing hyperexcitable cortex generate seizures and bilateral synchronous epileptiform discharges (Gloor et al., 1977, Gloor, 1979, Gloor and Fariello, 1988). In Gloor's generalised feline penicillin epilepsy model, bilateral synchronous spike and

slow-wave discharges are triggered by the normal inputs of the thalamic reticular system over a hyperreactive cortex. The physiological subcortical spindle-generating thalamocortical volleys provoke an increased number of action potentials in the hyperexcitable cortical neurons and secondary activation of the intracortical recurrent inhibitory pathway. This secondary intracortical inhibition generates an oscillation between enhanced excitation and enhanced inhibition that is manifested as a spike-slow wave in the EEG (Kostopoulos et al., 1981). This interaction between thalamic and cortical circuits in the epileptogenesis of GGEs has been supported by other authors (McCormick and Contreras, 2001). Furthermore, the cortical hyperexcitable state may be the result of either decreased intracortical inhibition (ICI) or excessive neuronal excitability. This could be attributed to faulty neurotransmission for which ion channel abnormities can be held accountable, as seen in some GGE with mutations in the sodium channels or the $GABA_A$ receptors (Berkovic and Scheffer, 2001).

Absence epilepsy is the only GGE with well-established in vitro and in vivo animal models that propose a disruption in the thalamocortical circuits as a causative mechanism for the generalised epileptiform discharges, for review (Badawy et al., 2009b).

In animal models of absence epilepsy, a disruption in $GABA_A$ mediated inhibition in the thalamic reticular cells makes them more responsive to excitatory postsynaptic potentials (EPSP), increasing their gabaergic inhibitory input over the $GABA_B$ receptors of the thalamocortical cells. This activation of $GABA_B$ mediated inhibitory postsynaptic potentials (IPSP) in the thalamocortical cells is crucial in the initiation of abnormal oscillatory rhythms in the thalamocortical circuits (McCormick and Contreras, 2001). Furthermore, projections from other distant brain structures also

modulate the thalamus or cortex, and disruptions on these neuronal networks may result in abnormal oscillated rhythms, which generate seizures (Snead, 1995).

Focal epilepsies

Mechanisms of seizure generation based on animal models of focal epilepsy and in vitro cellular studies of hippocampal slices suggest hyperactivity of multiple neurons followed by hypersynchrony. In those experiments, interictal spikes are likely generated by a propagated excitation between local networks when this excitatory input is not timely modulated by gabaergic inhibitory circuits (McCormick and Contreras, 2001, Berkovic and Scheffer, 2001, Engel, 1996).

C.1.1.3. Electrophysiological biomarkers of epilepsy

A disease biomarker is an objectively quantifiable structural and/or functional variable associated with the likelihood of developing a condition. In epilepsy, the variable would relate to the tendency to suffer epileptic seizures and develop chronic epilepsy.

Biomarkers are, to a great extent, used in medicine for diagnostic and prognostic purposes and for predicting treatment outcomes. Still, reliable epilepsy biomarkers have not been defined. It is well known that interictal discharges on the EEG are reasonably specific for epilepsy but their sensitivity is low and neither are associated with clinical outcomes. The structural lesions seen on MRI are not always epileptogenic, while many epilepsies have normal brain imaging (Kimiskidis, 2016). The discovery of reliable biomarkers for epileptogenicity associated with the presence, development, progression and/or severity of epilepsy would greatly improve the diagnostic and therapeutic approaches.

Several promising neurophysiological biomarkers have been recently proposed (Engel, 2011), including transcranial magnetic stimulation (TMS). TMS is a non-

invasive brain stimulation technique that, combined with electromyography (TMS-EMG), assesses motor cortical excitability and combined with electroencephalography (TMS-EEG), provides additional information on cortical reactivity, excitability and connectivity outside the motor cortex with a high temporal resolution (Sueri et al., 2018). Generalised and focal epilepsies have different pathophysiologic mechanisms, which would result in distinctive findings in the TMS studies (Macdonell et al., 2002).

TMS- EMG

Several studies have stated the value of TMS-EMG as a diagnostic biomarker of epilepsy. For instance, some TMS–EMG studies have identified alterations in motor cortical excitability associated with specific focal (Badawy et al., 2013c) and generalised epilepsy syndromes (Badawy et al., 2013d) as well as in asymptomatic siblings of epileptic patients (Badawy et al., 2013b). This is suggestive of a familiar trait involved in both focal and generalised epilepsies, which ultimate phenotypical manifestations would result from an interplay between genetic and environmental factors.

In GGE, different subtypes may display different TMS-EMG findings, likely related to distinct pathophysiology. For instance, some studies in GGE show a decreased motor threshold (MT) in comparison to controls. This finding was interpreted as an indicator of increased cortical excitability (Reutens and Berkovic, 1992). However, the Gianelli group reported a higher MT in absence epilepsy than in controls (Gianelli et al., 1994), suggesting that the cortical excitably may differ between different GGE types. Therefore, some reported TMS-EMG cortical hyperexcitability patterns might be syndrome specific, e.g., some studies in juvenile myoclonic epilepsy (JME) show a cortical excitability profile, evidenced by reduced MT and decreased long and short

intracortical inhibition (ICI), more pronounced than in the other GGE groups (Badawy et al., 2013d).

Several TMS-EMG studies in patients with GGE looking at impairment in GABA mediated cortical inhibition have been reported, assessing short and long interval intracortical inhibition (ICI, see section C.1.3.2.3) (Badawy et al., 2007). However, these studies have limitations, one of them being the failure to test the possibility of increased $GABA_B$ mediated thalamic inhibition, supported by the previously mentioned experimental animal studies (see section C.1.1.2). The paired-pulse TMS-EMG recovery curves' studies suggest that both $GABA_A$ and $GABA_B$ receptor-mediated inhibition is decreased in the neocortex in GGE (Badawy et al., 2007, Badawy et al., 2013b). Particularly TMS parameters suggesting the reduction in $GABA_A$ mediated inhibition (short cortical inhibition) have been supported by molecular studies showing $GABA_A$ receptor mutations (Baulac et al., 2001, Harkin et al., 2002, Wallace et al., 2001). In contrast, the TMS-EMG finding of an increased cortical silent period in untreated GGE (Macdonell et al., 2001) may support the hypothesis of increased inhibition driven by the thalamic pathways inputs as a secondary compensatory mechanism to prevent seizure initiation (Tassinari et al., 2003). For instance, this may be the case in GGE with tonic-clonic seizures, a possible explanation being a cortical origin of the seizure and thalamocortical regulatory inhibition of the spreading of ictal activity (McCormick and Contreras, 2001).

Focal epilepsies also display a distinctive response to TMS-EMG, showing hyperexcitability circumscribed to the affected hemisphere containing the epileptogenic focus (Badawy et al., 2013c) in contrast with the GGE in which the excitability disturbances are present bilaterally (Badawy et al., 2007). TMS-EMG studies suggest reduced GABA mediated cortical inhibition in the affected hemisphere

(Badawy et al., 2007) as a possible mechanism in focal epilepsy. This hypothesis has been further supported by in vitro intra-cellular studies showing loss of function in $GABA_B$ receptors in temporal lobe epilepsies (Deisz, 1999).

TMS-EMG changes in cortical excitability have also been associated with the preictal state in focal epilepsies after drug withdrawal (Wright et al., 2006), predicting the short-term occurrence of seizures and with the peri-ictal state, 24 hours before and after a seizure, showing syndrome specific changes in drug-naive focal and generalised epilepsies (Badawy et al., 2009a).

Studies in newly diagnosed and refractory epilepsy suggest that TMS may be an early biomarker of pharmaco-resistance in individual patients (Badawy et al., 2010, Badawy et al., 2013a) as persistent or progressively hyperexcitability following treatment indicates a poor response to the antiepileptic drug (AED), and conversely the administration of an effective AED reversed cortical hyperexcitability in patients that became seizure-free after treatment.

TMS-EEG

Regarding TMS-EEG as a biomarker of epileptogenicity, TMS-EEG studies performed in patients with long-standing focal epilepsy have found that EEG responses to single-pulse TMS stimulation, termed late responses, are of localising value to identify the epileptogenic zone and increase the diagnostic yield in focal epilepsy cases with a normal baseline EEG (Valentin et al., 2008). The late cortical responses to TMS evoked in the vicinity of the epileptogenic focus or regions functionally connected to the lesions (Shafi et al., 2015) are promising biomarkers of increased cortical excitability and pathological connectivity in focal epilepsies.

TMS-EEG studies in GGE reveal pathological states of enhanced excitability. The responses to single-pulse TMS stimulation after sleep deprivation were studied in patients with juvenile myoclonic epilepsy (JME) and in controls. The modulatory effect of sleep and sleep deprivation on TMS evoked potentials (TEPs) showed differences between JME and controls. The impact of sleep deprivation on the TEPs' amplitude enhancement and topographic distribution differed between JME and controls, as the JME group showed increased amplitude in the later components (P100, N190) in the anterior cortical regions. Overall, JME showed excessive cortical reactivity to TMS stimulation after sleep deprivation with a predominately anterior topographic distribution, and this enhancement was less pronounced in controls occurring in central and posterior areas (Del Felice et al., 2011).

Kimiskidis et al. studies suggested the presence of diffuse cortical hyperexcitability in patients with GGE. In this study, "high excitability" states were found in multiple locations during the interictal period associated with the generation of TMS-induced epileptiform discharges (EDs) (Kimiskidis et al., 2015).

In a later phase II study (Kimiskidis et al., 2017), the paired-pulse TMS-EEG responses studied with multi-level data analysis increased the diagnostic accuracy to discriminate patients with GGE epilepsy and their responsiveness to treatment.

C.1.2. The standard routine electroencephalography (EEG) as a diagnostic tool in epilepsy

C.1.2.1. Basic biophysical mechanisms of electroencephalography

The EEG records electrical activity from different areas of the cortex. The source of this electrical activity is the summation of extracellular currents generated by synchronised excitatory postsynaptic potentials (EPSP) and inhibitory postsynaptic potentials (IPSP), mainly from large pyramidal cells from cortical layers IV/V.

The well-established columnar organisation of the cortex plays a fundamental role in how these potentials manifest in the EEG recordings. The functional cortical unit is organised as a cortical column. Each cortical column contains parallel, radially orientated micro- columns and each of the micro-columns includes 80-100 neurons. The neurons inside each of these micro-columns behave as a synchronised functional unit receiving signals from deeper brain structures and also laterally connecting to neighbouring micro-columns. This anatomo-functional organisation allows synchronisation of extracellular postsynaptic potentials, which are volume conducted and subsequently recorded on EEG.

The synchronised inhibitory or excitatory postsynaptic activity results in an ionic exchange between neurons and their surroundings. The activity recorded in the EEG reflects electrical fluctuations in the extracellular space as the intracellular space is electrically insulated by the cellular membrane, and therefore intracellular currents are not visible with the surface EEG (Schomer and Lopes da Silva, 2017).

The extracellular potentials are the result of either excitation of the distal dendritic region (EPSP) or inhibition of the neuronal soma by inhibitory interneurons (IPSP).

When an EPSP is generated in the dendritic membrane, the depolarisation is caused by an influx of Na+ inside the neuron, provoking relative negativity in the adjacent extracellular space. Conversely, when the IPSP reaches the membrane of the soma of pyramidal neuron (upon excitation of the inhibitory interneurons), the entrance of Cl- causes a relative depletion of negative charges in the extracellular space near the cell body. For both EPSP and IPSP, anions flow to the positively charged area and cations flow to the negatively charged area, generating a current flow that goes from the source of origin of the potential (Beniczky and Schomer, 2020).

The dipole theory states that the EEG signals can be represented as single electrical dipoles with different orientations relative to the surface of the skull. The concept is based on a large number of cortical pyramidal neurons that, upon being synchronised, would constitute a single electrical dipole. As explained above in more detail, the voltage potentials between the dendrite's distal and proximal regions produce an electrical dipole and extracellular current flow. Due to passive volume conduction, these local field potentials travel through structures with conductive properties such as the skull, spinal fluid and skin to the surface, where they can be pickup by the EEG electrodes.

Approximately 7-10 square centimetres of synchronised cortical surface is required for the activity to be recorded in the EEG. With a higher degree of synchronisation, a smaller area could be involved in the EEG signal. The position and the orientation of the cortical sources in relation to the scalp determine the activity recorded on the surface. In the case of a source near the surface of the cortical gyrus, a simple radial cortical dipole with maximum negativity facing the scalp can be seen in the EEG area immediately above the source. When the source is generated in a complex gyrus like the Sylvian fissure, a similar dipole effect can be seen; as the complex gyrus has

activity in the base (going deeper into the sulcus) and also activity in the upper and lower sides (that cancel each other), the remaining activity is the one coming from the gyral base. This anatomical configuration results in a radial dipole with negativity towards the surface but with a smaller voltage reflecting a deeper source. If the source is in the interhemispheric fissure or in the anterior or posterior wall of the Rolandic fissure, the dipole is directed horizontally or tangentially to the scalp. Tangential dipoles can be misleading for source localisation as they have maximum positivity and maximum negativity with similar amplitude in both hemispheres. In the case of the interhemispheric fissure, the negativity is seen in the hemisphere contralateral to the source generator. For the Rolandic fissure, a source in the posterior wall points negativity towards the frontal areas and positively towards the posterior areas. The opposite is true for a dipole generator located in the anterior wall (anterior positivity and posterior negativity) (Beniczky and Schomer, 2020).

C.1.2.2. Technical aspects: The EEG machine

The incoming voltage signals are first recorded by the scalp electrodes. The standard routine EEG electrodes have to be safe as they are in contact with the skin. Also, they have to be electrically stable.

Non-polarisable small disks (10 mm diameter) of silver/silver chloride are commonly used. They are coupled with an electrolytic paste to create an electrode-based potential between the scalp and the metal. This steady-state electrode-skin potential is sensitive to the fluctuating voltages generation by the cortex underneath. The currents between the paste and the metal depend on the alternating voltages and electrode impedance in accordance with Ohm's law for alternating current (AC). V= I (current) x Z (impedance). Therefore, it is important to keep the skin impedances

relatively low (under 5000 Ohms) or equal, at least between electrodes (Beniczky and Schomer, 2020).

The EEG machine is comprised of three principal components: First, a differential amplifier to take in the incoming signal; second, an anti-alias filter combined with an analog to digital (A/D) converter and digital storage units and third, a display unit controlled by a system software that allows different display settings including montage, filter, sensitivity and time-scale.

In most modern EEG machines, each of the recording electrodes is referenced to the machine's common recording reference (CRR), and the difference between two electrodes is recorded by a high impedance input differential amplifier. As differential amplifiers record the difference between both recording electrodes, the output will be zero when both electrodes record the same signal (common-mode rejection or CMR). This is extremely useful to avoid external signals and reduce external noise.

However, differential amplifiers are not perfect, and for very high amplitude signals, there will be output even if both signals are identical. The quality of a differential amplifier is measured with the common-mode rejection ratio (CMRR), which is the ratio between output and input amplitudes when both input signals are the same. An ideal differential amplifier provides a very high gain for differential signals and zero gain for common-mode signals. The resulting signal of the balanced differential amplifier is an analog signal with a negative potential differential going upwards and a positive potential differential going downwards.

This analog signal leaving the amplifier is processed by an analog anti-aliasing filter. This filter attenuates the amplitude of the signals with an insufficient sampling rate following the Nyquist Theorem (the highest resolution of an analog signal that can be

digitally seen is half of the sampling rate). Following the anti-alias filtering, the signal is sampled digitally by the analogue to digital converter and placed into the digital storage unit as the value given by the difference of the potentials between the recording electrode and the CRR. Subsequently, in the display unit, the system software allows to manipulate the amplitude, frequency range and time scale of the signals and displays the EEG channels using various arrays (montages) (Schomer and Lopes da Silva, 2017).

C.1.2.3. EEG recording and reviewing principles

The EEG electrodes visualise the electrical activity as the voltage difference between two electrodes, showing an upwards negative polarity and a downwards positive polarity (conversely to the standard convention in physics and mathematics). Therefore, an EEG channel assesses the difference in voltage between two different areas of the scalp. This voltage difference changes over time, reflecting the fluctuation of the electrical activity in the area of recording.

In clinical practice, the EEG-electrodes are placed on the scalp according to a standardised position system. The most widely implemented is the 10-20 international system. The International Federation of Clinical Neurophysiology (IFCN) electrode array recommendation is based on the 10/20 system plus six additional sub temporal electrodes. The 10/20 system uses the nasion, inion, and the two preauricular points as anatomical landmarks to draw horizontal and vertical lines wherein the electrodes are placed (Seeck et al., 2017). The electrodes' names are based on the region they are placed: Fp (Frontopolar), F (frontal), C (central), T (temporal), P (parietal), O (occipital). These names are followed by an underscore number. The odds numbers indicate that the electrode belongs to the left hemisphere, the even numbers to the

right hemisphere and the underscore letter Z indicates that the elected placement is in the midline. The smaller the number, the more centrally located the electrode and vice versa. The IFCN is a 25-scalp electrode array, and the 10-20 system is a 19-electrode array.

The scalp electrodes are connected to each other in various arrays called montages. Depending on the reference used, the montages are classified into two main groups: bipolar and referential. If all the electrodes are connected to the same common reference, the montage is called referential. If the electrodes are connected two by two, each electrode reference changing in a systematic way, the montage is called bipolar. In a bipolar montage, the pairs of electrodes are organised in longitudinal chains going from front to back (longitudinal bipolar montage) or from left to right (transverse bipolar montage). In the referential montage, the common reference can be another single electrode (the Cz or the linked ear lobes are the most commonly used) or a computed reference. The computed common reference is calculated as the common average of all the recording electrodes. As the scalp electrodes have variable positive and negative potentials, the summation of all these potentials cancels each other, resulting in an approximation to a zero potential. Kirchhoff's second law states that the sum of the potential differences around any closed loop is zero. Thus, the common average reference is similar to an indifferent reference (Beniczky and Schomer, 2020).

A routine EEG usually lasts for approximately 30 minutes (awake EEG) or for 60-90 minutes to record a period of sleep (sleep EEG). Sleep deprivation or sleep-inducing drugs can facilitate the sleep EEG. Apart from sleep, other activation manoeuvres such as hyperventilation and photic stimulation are performed to potentiate the diagnostic yield of the EEG.

C.1.2.4. EEG and epilepsy

Nowadays, scalp EEG is still the most widespread method to support the diagnosis of epilepsy. The EEG helps to support the diagnosis and classification of epilepsy. Furthermore, the ILAE establishes the EEG as instrumental for the classification of epilepsies (Scheffer et al., 2017). However, the EEG provides limited aid for the aetiology classification of epilepsy. Although there are some ictal EEG patterns considered pathognomonic for epilepsy diagnosis, seizures are relatively infrequent in an outpatient routine EEG recording setting, and the diagnosis of epilepsy is mostly based on interictal activity. The same EEG pattern may be present in epilepsies with various causes, and on the other hand, syndromes within the same aetiological group, for instance, GGE, have different electrographic features (Koutroumanidis, 2017).

C.1.2.4.1. Sensitivity of the EEG in epilepsy

The most reliable EEG indicator to support the diagnosis of epilepsy is the presence of epileptiform discharges (see further explanation in C.1.2.5.). Approximately only 26-55% of the routine awake EEGs recorded in patients with epilepsy show interictal epileptiform discharges (Smith, 2005). Several factors influence the presence of epileptiform discharges in the EEG. The epilepsy type, focal or generalised, and the location of an epileptogenic zone are relevant. The yield of the interictal awake EEG is higher in patients with generalised seizures than in focal epilepsies. Some authors report abnormal EEG in approximately 31 % of patients with focal seizures, in contrast to 77% in patients with generalised seizures (Delil et al., 2015). Specifically, the yield is low in patients with focal epilepsy arising from mesial or basal cortical regions, which are remote from the scalp electrodes. This is the case in mesial frontal lobe epilepsy which often has normal interictal EEGs (Pillai and Sperling, 2006). The yield of

recording epileptiform discharges may increase to 80-92 % when at least two sleep EEGs are performed (Salinsky et al., 1987). Besides sleep recordings, the chances of recording epileptiform discharges increase when the EEG is performed soon after the event.

C.1.2.4.2. Specificity of the EEG in epilepsy

Although the recording of epileptiform discharges is uncommon in the normal population (0.2-0.3%), in non-epileptic patients with a previous history of a neurological condition or intracranial neurosurgery, these EEG abnormities are more frequently seen (5-20%). In those cases, the findings should be interpreted with caution as they do not necessarily convey the diagnosis of epilepsy (Alarcón, 2012).

Furthermore, seizure frequency and severity, disease prognosis or responsiveness to treatment are not associated with abnormalities in the EEG. The interictal EEG not always shows abnormalities in epileptic patients; however, repeatedly normal EEGs should doubt the diagnosis.

C.1.2.4.3. The value of the interictal EEG in epilepsy

For the EEG interpretation, both the background activity and the paroxysmal abnormalities (see below 1.2.5.) have to be considered.

The assessment of the background activity gives certain clues to aid in the epilepsy classification. For instance, the GGE display normal background activity. In contrast, focal epilepsies often show focal slowing suggesting an underlying structural abnormality. Generalised epilepsies due to an underlying organic or metabolic pathology have a diffuse slowing of the background. Diffuse slowing may also result from the iatrogenic effect of antiepileptic drugs (AEDs). Because this AED induced slowing is dose-dependent, the AED dosage should be documented to avoid

overinterpretation of the slowing. An increase in fast rhythms (beta activity) also occurs with certain AEDs such as barbiturates and benzodiazepines.

The paroxysmal abnormalities are classified as focal or generalised, and they will be described in more detail in section 1.2.5.

Generally speaking, an interictal EEG with generalised epileptiform discharges without slowing of the background suggests GGE epilepsy. Additional EEG features such as the presence of photosensitivity further support the diagnosis. The presence of focal epileptiform discharges in a particular region suggests focal epilepsy likely arising from these areas. If additional focal slowing is present, a presumed symptomatic cause has to be considered, and neuroimaging is advised. Genetic focal epilepsy is suspected in patients of certain age groups with a normal background activity and focal epileptiform discharges of certain topography characteristics. These EEG features have to be judged in the context of the clinical history. Lastly, focal, multifocal and/or generalised epileptiform discharges accompanied by diffuse background slowing suggest symptomatic generalised epilepsy.

Overall, the interictal EEG assists in supporting the diagnosis of epilepsy. However, the interictal EEG alone is insufficient to fully prove or disprove the diagnosis of epilepsy. Still, EEG is an important tool to identify or rule out specific epilepsy syndromes (e.g., childhood absence epilepsy is unlikely if an appropriate hyperventilation manoeuvre fails to evoke an absence seizure).

C.1.2.4.4. The value of the ictal EEG in epilepsy

Ictal EEG recordings are the EEGs recorded during seizures. These convey more information than the interictal recordings to confirm or refute the diagnosis of epilepsy. However, they are more difficult to obtain in an outpatient routine EEG setting as,

generally, seizures are infrequent. Still, seizures may occur during the standard awake or sleep EEG recordings in certain syndromes with a high frequency of seizures (such as childhood absence epilepsy or symptomatic generalised epilepsies). Certain seizures types always show characteristic ictal EEG findings (e.g., typical absence seizure showing three times per second (3 Hz) spike and slow-wave discharges as the hallmark of childhood absence epilepsy), but other seizures types (e.g., focal onset aware seizure of frontal or temporal origin) may also present without EEG changes, or with EEG artefacts due to muscle activity. Other pitfalls of the ictal EEG concern seizure classification. For instance, although the focal impaired awareness seizures have a focal origin, only in approximately 30% of these seizures the scalp EEG shows a clear focal onset, while diffuse bilateral changes are seen in the rest. This is due to the fact that extensive cortical recruitment has occurred by the time that the changes appear on the scalp EEG.

Overall, ictal EEG changes occurring during a clinical episode confirm the epileptic nature of the event. However, most but not all epileptic seizures are accompanied by ictal EEG changes. Focal myoclonic seizures and focal onset aware seizures do not always show ictal changes as the cortical area generating these seizures is often small and/or deeply embedded in the lobar structures (e.g., temporal lobe, hippocampus-amygdala complex or mesial frontal). On the contrary, if the EEG remains normal during an episode of unresponsiveness or generalised convulsions, the event is considered to be not epileptic in nature. In order to suffer from loss of consciousness or generalised convulsions, significant cortical recruitment is needed, and this unambiguously would evolve into EEG changes (Alarcón, 2012).

C.1.2.5. EEG analysis in epilepsy

C.1.2.5.1. Visual analyses of the spontaneous waveforms

There are EEG features strongly associated with epilepsy, and in some epilepsy syndromes, the EEG provides an electrographic signature.

Apart from the background activity, the EEG also records occasional brief and short-lasting (usually less than a second or two) waveforms called paroxysmal events. These are believed to be the result of sudden synchronisations of neuronal activity. The paroxysmal events can be physiological, such as the sleep graph elements (vertex sharp waves and K complexes) or abnormal as the epileptiform discharges.

Paroxysmal abnormalities in epilepsy

The interictal paroxysmal abnormalities are generated by sudden neuronal synchronicity. They require at least 6 square centimetres of synchronous cortex to be seen in the scalp EEG (Tao et al., 2005).

The paroxysmal abnormalities are classified by morphology and duration in:

a) Sharp waves: Generally negative, triangular-shaped pointed waves with a blunted peak and a duration longer than 70 milliseconds (70-200 milliseconds).
b) Spikes: Sharper triangular-shaped waves, shorter than 70 milliseconds in duration (20-70 milliseconds). The peak is also generally negative in epileptic conditions, and the amplitude is variable.
c) Polyspikes: runs of spikes lasting for several hundreds of milliseconds.
d) Spikes and polyspikes may be followed by a slow wave, constituting repetitive patterns known as spike-and-wave or polyspike-and-wave complexes.

Spikes, polyspikes, and spike-and-wave complexes are commonly described as epileptiform discharges because they are frequently seen in patients with epilepsy.

C.1.2.5.2. Quantitative analysis of the EEG

The quantitative EEG (qEEG) analysis of the digitised EEG is an extension of the visual EEG. This procedure processes the recorded EEG data by implementing various mathematical algorithms for spectral analyses, such as the Fast Fourier Transform (FFT) or the Wavelet transform. These algorithms transform the digital EEG signal into numerical data that represents the power in each frequency band (Kaiser, 2005).

Some studies showed increased absolute power in all frequency bands in epileptic patients in comparison to healthy controls (Livint Popa et al., 2020, Tedrus et al., 2019). Other studies have reported increased delta, theta and beta power in epilepsy in general and a characteristic increase in gamma power in GGE that was not seen in focal epilepsy (Willoughby et al., 2003). Santiago et al. group reported an increase in absolute power (AP) of alpha, beta, delta and, to a lesser degree, theta bands in the background EEG of JME patients (Santiago-Rodriguez et al., 2008). In contrast, a recent study by the same author stated an increase in the beta band AP and a decrease in delta band AP as the most commonly found qEEG analysis abnormalities in JME patients. This study considered alpha band findings, particularly the decreased alpha AP as a possible pharmacological effect (Santiago-Rodriguez and Zaldivar-Uribe, 2021). Increased alpha, theta and mostly delta AP was reported in GGE, being these features more pronounced in JAE than in JME patients (Clemens et al., 2000). In focal epilepsies with abnormal interictal EEGs, Drake et al. study showed decreased high frequency-low frequency power ratios in the side of the focus compared to non-epileptic controls. This seemed in keeping with increased slow activity on the focus

side. In focal epilepsy patients with normal interictal EEGs, the high frequency-low frequency power ratio was also reduced to a lesser degree (Drake et al., 1998).

Other studies analysing interictal EEG recordings also described spectral power differences between epileptic patients and non-epileptic controls, with specific results in the alpha and delta frequency bands. Reduced values in the quantitative measures in the alpha band were described in epileptic patients (Miyauchi et al., 1991), and some authors proposed the reduction of alpha power as a prospective biomarker for epilepsy detection (Larsson and Kostov, 2005, Pyrzowski et al., 2015). Furthermore, some authors capitalized on the increased delta power and reduced alpha power seen in the resting interictal EEG recordings of epilepsy patients to implement an epilepsy detection machine learning algorithm (Buettner et al., 2019, Rieg et al., 2020).

C.1.3. Transcranial magnetic stimulation (TMS) general principles

C.1.3.1. Physics of TMS

Transcranial magnetic stimulation (TMS) is a non-invasive brain stimulation modality pioneered by Barker and colleagues in 1985 (Barker et al., 1985) which relies on the electric current generated by a magnetitic field to induce depolarization in selected neuronal populations in the brain tissues.

C.1.3.1.1. Principles of electromagnetism

The magnetic stimulation is based on Faraday's law of electromagnetic induction. An electric current passing through a coil generates a magnetic field, and this changing magnetic field would subsequently induce an electric current flow in a conducting media like the human brain.

A very simple model of electromagnetic induction may consist of two coils placed together. An electrical current in one of the coils generates a changing magnetic field (BF), resulting in an electric field (EF) which in turn will induce a second electrical current (eddy current) in the second coil. This second current has an opposite direction to the primary current in the first coil (Figure 1.1).

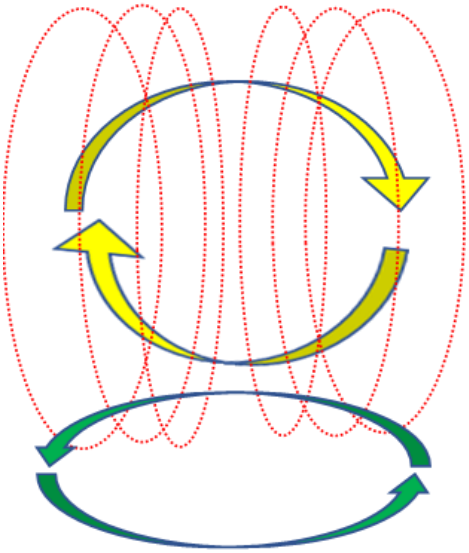


Figure 1.1. Schematic representation of electromagnetic induction in a simple transformer consisting of two loops: Loop 1, represented by the yellow circle arrows, shows the electrical current that generates a magnetic field (red ellipses), and loop 2, represented by the green arrows, shows the secondarily induced electrical current (eddy current).

The energy stored in the BF is measured in joules (J), and it is proportional to the primary current and the inductance of the coil. The strength of the EF is measured in volts (V), and both the EF and the secondarily generated current are proportional to the rate of change per time in the magnetic field (d*B*/d*t*).

The basic magnetic stimulator circuit consists of a capacitor and an inductor (the stimulation coil) connected by a switch. The capacitor, once charged with a high voltage, discharges in the coil once the switch is closed (Figure 1.2).

A single transcranial magnetic stimulation (TMS) pulse may reach the order of hundreds of joules (J) of energy level. The large energy and very high power required for TMS limited the earlier systems in terms of the pulse duration and waveforms delivered.

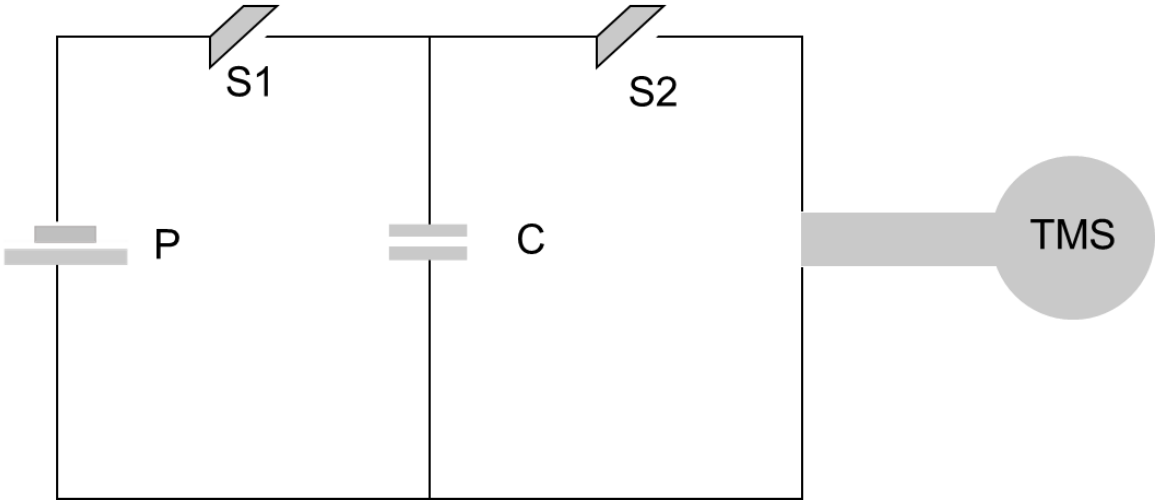


Figure 1.2. Schematic representation of a basic magnetic stimulator showing an electrical power source (P) that charges the capacitor (C) upon closure and reopening of the switch (S1). The capacitor (C) will discharge into the TMS coil (TMS) once the switch (S2) is closed.

C.1.3.1.2. Monophasic versus biphasic stimulation

At the beginning of a TMS pulse, the energy is stored in the capacitor. After the switch closes and the capacitor discharges, the current flows and the capacitor's electrostatic energy is transferred to the magnetic field of the coil.

There are monophasic stimulators that generate a monophasic current of single polarity and biphasic stimulators which generate a sinusoidal cycle.

The biphasic stimulator design is more efficient for rapid repetitive stimulation due to the lack of an internal resistor that dissipates the energy as heat. Instead, the energy is recycled back to the capacitor. This prevents the rapid heat-up of the system during high frequencies repetitive pulses, in contrast to the use of a monophasic system.

The monophasic pulse has a sharp initial quarter of the cycle where the circuit voltage is zero, the current is maximum, and the energy is moved to the coil's magnetic field.

Following this, the current dissipates through the resistor and does not recharge the capacitor. For the simplest monophasic stimulators, the capacitor charge is lost in the resistance, making the device less efficient. In contrast, for the biphasic system, there is no resistor, and after discharging, the current flows in one direction, reaching its peak value and then there is a second phase where the current reverts to the opposite direction. During the second phase, most of the current returns to the capacitor. This generates a biphasic pulse where the current is zero halfwaves in the cycle, and the energy is back to the capacitor but at a reverse voltage. Three quarters into the cycle, the voltage is zero, and the current is maximal in the opposite direction; by the end of the cycle, the current is zero, and the surplus energy is back to the capacitor in its initial polarity. The current will flow back and forwards between the capacitor and the coil until the energy eventually dissipates in the system. This allows for the recycling of some of the energy getting ready for a subsequent pulse.

The later components of the biphasic pulse may be the more effective for stimulation. Biphasic pulse stimulation renders lower values in the motor threshold than the monophasic pulse. It has been suggested that the last three-quarters of the biphasic pulse cycle contribute to these lower motor threshold values (Sommer et al., 2006). The biophysical mechanisms underlying this observation are still not fully understood, although some studies have demonstrated a difference in the D waves and I wave (see C.1.3.1.5 for further details) in epidural recordings obtained with biphasic versus monophasic stimulation. This suggests that the pattern of cortical activation varies with different stimulus waveforms (Di Lazzaro et al., 2004).

In single pulse machines, the pulse configuration is monophasic for the vast majority. For the rapid repetitive stimulators, the pulse configuration is biphasic for energy efficiency as the energy generated by each pulse helps to produce the next. The

stimulation with a monophasic pulse has the advantage of a lower click noise and lower heat release in compassion to the biphasic. The biphasic pulse is shorter in duration and more efficient but less accurate than the monophasic pulse. This is the consequence of the reversal of the current in the opposite direction during the second phase that stimulates different cortical elements than in the initial phase (Groppa et al., 2012).

C.1.3.1.3. TMS coils

The electric current in the stimulation coil generates a magnetic field, and this varying magnetic field induces an electric current flowing in the opposite direction, called eddy current, in adjacent conductors – such as human tissues and the brain. The geometry of the coil (shape, size) and the coil's orientation determines the characteristics of the magnetic field and the direction of the induced current (Cohen et al., 1990).

Circular TMS coils

In the circular coils, the current induced in the tissue is close to zero on the central axis and smoothly increases to a maximum on the outer edge under the circumference of the coil (Figure 1.3). During the stimulating phase, the current induced in the tissue flows in the opposite direction to the current in the coil. Most circular coils have good penetration to the cortex, but they lack focality as the fields are induced over a wider area. These properties make the circular coil useful when the target for stimulation is uncertain. The area of stimulation could be reduced by placing only one edge of the coil in contact with the scalp, but this manoeuvre would drastically compromise the efficiency of the stimulation.

Double coils (Butterfly or Figure of Eight coils)

An important development in coil design was the introduction of the double coil, where two windings were placed side by side in a figure of eight. The main difference over circular coils is that the tissue current is maximal in the centre, where the figure of eight crosses and the two electric field loops are superimposed maximally under the junction, providing more accurate localization (Figure 1.3). However, the penetration of the induced EF is more limited than with the circular coil, and the stimulation with the figure of eight coil is easily compromised by minimal changes in the coil position (see section C.1.3.1.4 for further details).

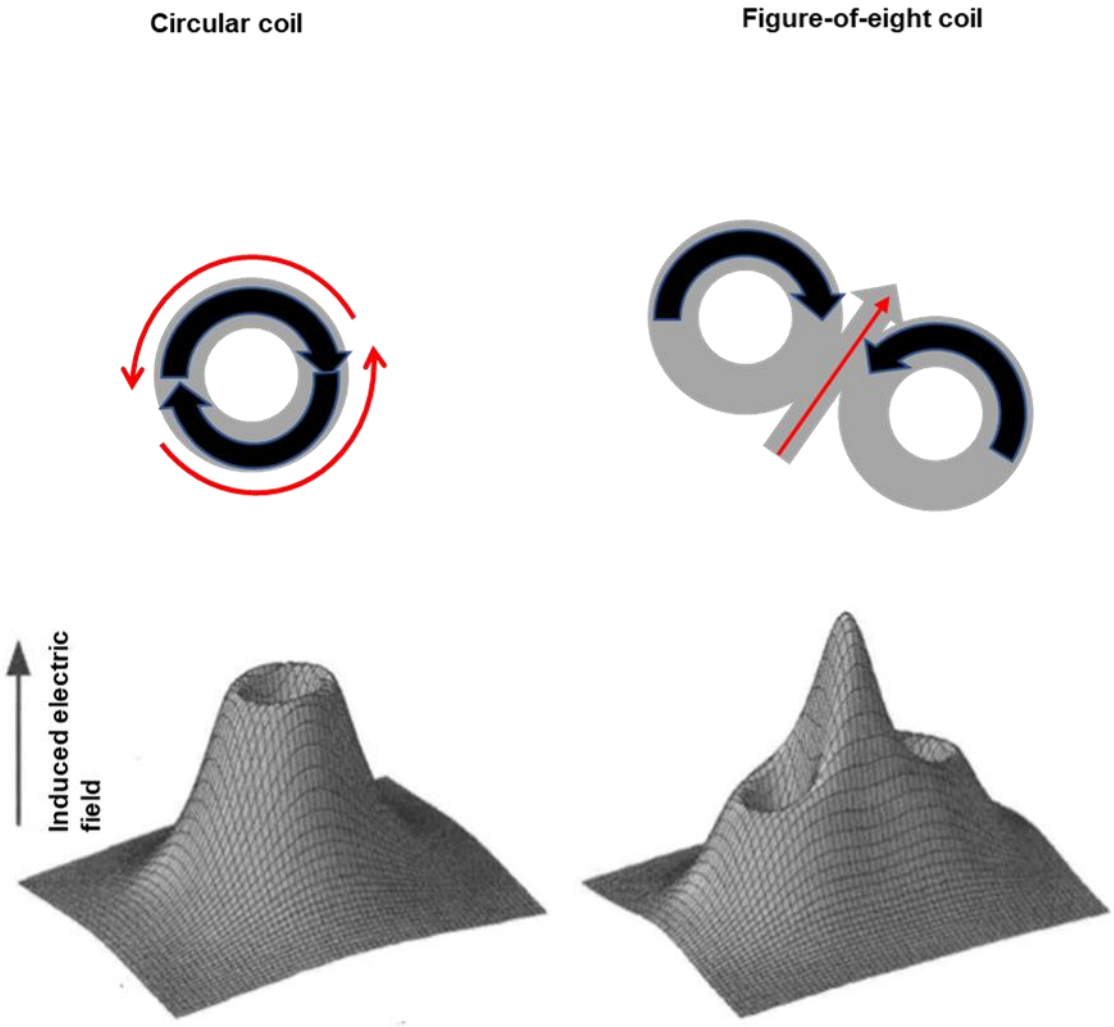


Figure 1.3. The induced electric field of a 90mm circular coil (left) and a double 70mm coil (right). The induced currents in the underlying tissues are shown in the form of concentric circles whose diameter is reduced with increasing depth. The black arrows represent the electric field (EF) induced in the coils by the magnetic field (BF). The red arrows represent the direction of the EF generated in the conductive tissue (modified from Magstim User Manual and Cohen et al.).

C.1.3.1.4. Biophysics of magnetic stimulation. The brain as a conductor.

The magnetic field does not cause neuronal depolarization directedly. This occurs as a consequence of an electric field (EF) secondarily generated in the brain by magnetic stimulation (Hallett, 2007, Hallett, 2000). This induced EF exponentially decreases as the distance from the coil increases (Epstein et al., 1990, Eaton, 1992, Maccabee et al., 1990). The EF and generated currents have a parallel orientation to the scalp in contact with the magnetic coil and the cortex underneath (Tofts, 1990, Cohen and Cuffin, 1991).

There is minimal attenuation of the TMS magnetic field when it penetrates the scalp and skull tissues. This allows the TMS to generate a secondary current in the brain sufficient to stimulate the cortical neurons without inducing a high intensity painful electrical current in the skin and making the TMS an effective and well-tolerated stimulation technique.

To gain insight regarding the TMS induced electrical activity in the brain, it is worthy to consider the possible differences in conductivity reflecting the white and grey matter organization. The brain and its basic components, white matter, grey matter, and cerebral spinal fluid, constitute an inhomogeneous conductor, but the differences in conductivity between white and grey matter are small. Considering that the differences in conductivity in the brain tissues are small, the brain can be considered as a homogeneous spherical conductor during magnetic stimulation, and a simple homogenous head model could estimate the TMS induced electric field and currents (Davey et al., 2003).

In a homogeneous conductor as the brain tissue, a round magnetic coil placed tangentially to the surface would induce an EF in the opposite direction to the one in

the coil. The EF is maximal under the winding and minimal toward the centre of the round coil. A tangentially orientated figurate of eight coil induces two electrical fields, which will superimpose the coil junction where the EF is maximal. The skull is the outer part of the sphere and has a zero-current density as its conductivity is 100 times smaller than the brain. When the current flows from a lower to a higher conductivity area, the interphase behaves as a virtual cathode. In the geometric model of the head, the TMS induced electric field is always parallel to the scalp and the radial field in the perpendicular direction is zero; therefore, the induced electric field at the centre of the head falls to zero as in the centre, all directions are radial. The radial fields, as a result of variations in conductivity, are insignificant; as was mentioned before, the differences in conductivity in the brain tissue are small.

The TMS stimulation is influenced by spatial parameters: the coil position and orientation in the head, the coil geometry and stimulation paraments: pulse intensity, the strength of the voltage in terms of the devices' maximum stimulation output (MSO) and pulse shape (monophasic, biphasic). These factors and the tissue conductivity determine the current distribution and the induced EF (Richter et al., 2013).

Specifically, to optimize the stimulation with the figure of eight coil, both the coil orientation (posterior-anterior, anteroposterior and mediolateral) and the coil angle in reference to the sagittal plane of the head are important. The interaction of the induced EF and the cortex anatomy defines the effective EF as postulated by Fox in his cortical column cosine model of TMS efficacy (Fox et al., 2004). Fox's model focuses on the orientation of the cortical columns and the orientation of the pyramidal cells' axons relative to the EF. The effective EF is parallel to the columns (normal to the cortical surface) as the pyramidal axons are parallel to the cortical columns' axis. More advanced models take into consideration a more complex interaction with the

convoluted anatomy of the cortex. For identical coils' EF, the current induced in the cortex depends on the coil's orientation relative to the gyri-sulci anatomy (Thielscher et al., 2011).

In addition to cortical excitation, direct axonal excitation at the subcortical white matter is also a proposed mechanism of TMS activation. In general, an axonal membrane would be more readily depolarized by steep gradients of the EF, for instance, when an axon bends, resulting in an abrupt change in the EF (Ruohonen and Ilmoniemi, 1999). The responsiveness to TMS cortical excitation or subcortical excitation may be influenced by factors like differences in the cortical and axonal excitability thresholds or variations in the gyral geometry. Still, fundamental questions regarding the neural substrate of the TMS activation remain, and the mechanics involved in the stimulation parameters modifying the neural response remain elusive.

C.1.3.1.5. Neurophysiology of TMS. Mechanism of generation of TMS evoked responses.

The TMS evoked responses rely on the functional state of the cortex and the locally induced electric field, which relies on the properties of the magnetic pulse (waveform and intensity) and the position and orientation of the coil.

The TMS induced electric field causes membrane depolarization as a result of the ion flow, which generates action potentials, trans-synaptic transmission and excitatory or inhibitory postsynaptic potentials. This makes TMS a sensitive measure of both the excitatory and inhibitory functions of cortical motor neurons, providing non-invasive clinical measurements of neuronal excitability (Macdonell et al., 2002).

Suprathreshold stimulation activates both trans-synaptic and direct axonal pathways, while lower intensity TMS activation is for the most trans-synaptic (Edgley et al., 1990).

Both excitatory and inhibitory interneurons are activated, the proportion of which would depend on stimulus intensity.

The electric field parallel to the surface of the cortex preferentiality stimulates the interneurons located in layers two and three.

The TMS evoked responses are recorded with two main techniques: electromyography (TMS-EMG) and electroencephalography (TMS-EEG).

TMS-EMG:

The cortical reaction to TMS stimulation was initially studied over the motor cortex, assessing brain response with surface EMG and epidural recordings.

The epidural recordings suggest that the intensity and direction of the TMS stimulation influence the neuronal substrate activated in the motor cortex. This activation translates into direct and indirect waves. The direct waves (D-waves) result from the direct excitation of the pyramidal cells' axons, while the indirect waves (I-waves) are generated by indirect trans-synaptic activation of the pyramidal cells (Lemon, 2008). The I-waves are subdivided based on their latencies in early I-waves (I1-waves) and late I-waves (I2, I3 waves). A lateral to medial EF direction generates D-waves first. The posterior to anterior EF direction generates an I1 wave that subsequently will be followed by late I-waves as the stimulus intensity increases. With the anterior to posterior direction, I3 waves are recorded at low stimulus intensities, followed by I2, I1 waves as the stimulus intensity increases (Di Lazzaro et al., 2001, Di Lazzaro et al., 2012). Although the mechanisms for I-waves generation are still not fully understood (Ziemann, 2020), the different current direction determined by the TMS orientation seems to activate separate cortical circuits involved in the D wave, and I wave generation (Ni et al., 2011) (Figure 1.4)

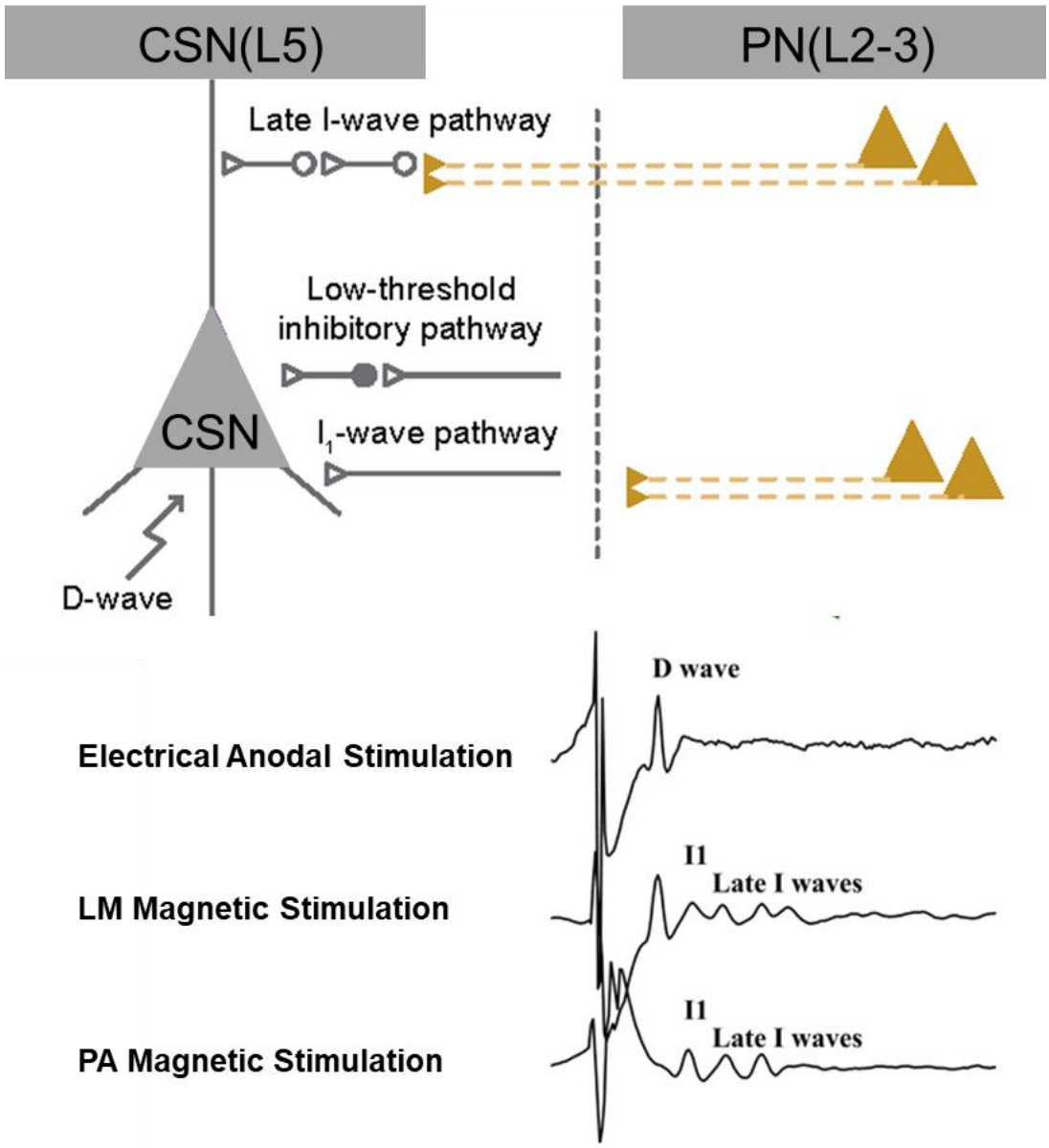


Figure 1.4. Schematic representation of the D-wave and I-wave generation. The D-waves are the result of direct excitation of the axonal hillock, while the I-waves reflect transsynaptic activation. The low threshold inhibitory pathway involves GABAergic interneurons involved in the modulation of the late I-waves. CSN, corticospinal neuron, PN, pyramidal neuron, LM, lateromedial, PA posteroanterior (modified from Lemon, 2008 and Di Lazzaro 2012)

TMS-EEG:

The combination of transcranial magnetic stimulation with electroencephalographic recordings has the advantage to measure the neuronal activity elicited by the magnetic stimulus with a high time resolution. This modality allows assessing the state of many cortical regions, besides the motor cortex, in addition, to providing information regarding functional connectivity between separate regions. The TMS evoked EEG responses convey information about both the local reactivity of the stimulated areas and connectivity (Ilmoniemi et al., 1997) as the TMS induced neuronal excitation can spread ipsilaterally and contralaterally from the stimulated areas (Ilmoniemi et al., 1999, Ilmoniemi and Kicic, 2010).

A more detailed explanation of the TMS-EEG responses and the diverse applications of TMS-EEG will follow in section C.1.3.3 (TMS-EEG parameters).

C.1.3.1.6. Technical challenges of combined transcranial magnetic stimulation and electroencephalography (TMS-EEG)

In the first attempts to record TMS evoked EEG responses in 1989, one hemisphere was stimulated and transcallosal responses at 8.8-12.2 milliseconds latency were recorded contralaterally (Cracco et al., 1989). Other experiments elicited EEG responses following cerebellar TMS stimulation (Amassian et al., 1992). These early attempts were limited to just a few electrodes distant from the stimulus due to the large artefacts generated by the TMS induced electric field. These electric fields may affect the electrode leads but also the electrode-electrolyte interface causing polarization and drift generated artefacts difficult to disentangle from the physiological responses.

The principal technical challenge is that TMS can provoke a large DC artefact that saturates the EEG amplifier.

Also, the type of recording electrodes used during TMS-EEG has to be considered since the changing magnetic field generates currents in the electrode, "eddy currents," causing a force proportional to the TMS intensity and to the thickness, diameter and conductivity of the electrode (Roth et al., 1992). This force may cause electrode movement resulting in artefacts (Virtanen et al., 1999). Furthermore, the electrode's currents generate Ohmic heating that potentially could cause discomfort and, in extreme conditions, such as a large diameter electrode, a high-intensity TMS stimulus or a high number of pulses, could result in burning skin lesions.

In order to prevent the interference of the TMS artefacts with the EEG biological signals, different steps can be considered. The most important technical development in TMS-EEG was the TMS compatible EEG amplifiers. This has led to the development of high-quality TMS-EEG recordings. To make this possible, different hardware modifications have been implemented. A slew rate limited amplifier developed by (Ives et al., 2006) prevents saturation during the TMS pulse, and the artefact is limited to a maximum of 30 milliseconds of duration. The amplifier does not eliminate the artefact generated by the magnetic pulse but remains operative, providing a clear EEG signal a few milliseconds after the pulse. Another solution is a sample and hold circuit that erases the time periods in which the TMS pulse is delivered, blocking the artefacts from TMS induced voltages in the leads (Virtanen et al., 1999, Ilmoniemi and Kicic, 2010).

It is important to ensure a low contact resistance between the scalp and the EEG electrodes for a reliable electrical connexion. An appropriate input impedance in the amplifier ensures signal quality in the recording.

Regarding the eddy currents electrode artefacts, cutting a slit in the electrode (from O-shape to C-shape) reduces the heating effect and also the artefact generated by the DC shift (Rossi et al., 2009)**.**

C.1.3.2. TMS-EMG parameters and physiological mechanisms underlying TMS-EMG parameters

C.1.3.2.1. TMS-EMG general introduction

Traditional TMS-EMG protocols rely on studying the hand muscle potentials evoked by the stimulation of the contralateral motor cortex, namely motor evoked potentials (MEP), as an indirect measure of global cortical excitability (Kobayashi and Pascual-Leone, 2003). Some of these values are obtained with single pulse stimulation, while the others would need a preceding subthreshold or suprathreshold conditioning stimulus (CS) delivered before each subsequent test stimulus (TS) by means of the paired-pulse stimulation technique applied at variable interstimulus intervals (ISIs). To that effect, the following EMG derived parameters are commonly quantified for the evaluation of cortical excitability:

C.1.3.2.2. Single-pulse TMS parameters: Motor Threshold (MT), cortical silent period (CSP)

Motor Threshold (MT)

The motor threshold (MT) is the minimum TMS stimulus intensity needed to evoke a robust muscle response and reflects the excitability of the cortical motor neurons. The cortical excitability is highly variable amongst different individuals, making it necessary to establish the threshold in the different participants before starting a TMS study. This is due to the fact that the subjects have to be stimulated with biologically equivalent intensities in order to compare the TMS evoked responses. It is also important to

obtain an adequate motor threshold to ensure comfort and safety during the procedure.

Therefore, several methods may be considered to find a technique both practical and reliable to determine the MT.

There are several methods to calculate the MT. These include:

1. Relative frequency methods
    1.1. Rossini-Rothwell Methods
    1.2. International Federation of Clinical Neurophysiology (IFCN) standardised algorithm
2. Mills and Nithi Method or the two-threshold method
3. Adaptive methods based on a probabilistic approach.
    3.1. Awiszus' threshold hunting algorithm
    3.2. Bayesian Adaptive Method

1. Relative frequency methods.

The relative frequency methods based on the Rossini criterion define the MT as the minimum stimulus intensity required to elicit an MEP in five out of ten consecutive trials. A positive MEP requires a minimum peak to peak amplitude of 50 microvolts at rest or 200 microvolts during tonic contraction (Rossini et al., 1994).

The Rossini method starts at a non-specified subthreshold intensity which will be gradually increased by 5% steps of the maximum stimulator output (MSO) until MEPs appear in 50% of 10 to 20 trials. A revised version of this procedure, the Rothwell

method, starts with a suprathreshold intensity which is decreased in 2% or 5% steps of the MSO until the 50% MEP induction is no longer obtained (Rothwell et al., 1999).

The IFCN offers a standardised algorithm, which is based on a modified version of the Rossini-Rothwell relative frequency criterion. The experiment starts with a subthreshold intensity which will be gradually increased by 5% steps of the MSO until MEPs appear in each trial. Subsequently, the intensity will be gradually decreased by 1% MSO steps until the 50% MEP induction would no longer be achieved, with the responses elicited in less than five out of ten consecutive trials. The resulting intensity value plus one is considered the MT (Groppa et al., 2012).

2. Mills and Nithi's two-threshold method

This method considers the MT as the arithmetic mean of the upper threshold (UT), defined as the lowest intensity resulting in the presence of 10 positive MEPs out of 10 trials (probability 1 to elicit an MEP) and the lower threshold (LT) defined as the highest intensity with the absence of MEPs out of 10 trials (probability 0 to elicit an MEP).

The lower motor threshold is calculated by starting the stimulation at 20% of the maximum stimulator output (MSO) and increasing the intensity by 10% MSO increments until at least 1 in 10 stimulations provokes a motor response. The intensity is then decreased by 1% steps until the stimulation does not produce a response (0 out of 10 trials). The upper threshold is calculated by starting off at the lower threshold and increasing by 1% increments until 10 MEPs out of 10 stimulation trials are accomplished (in some protocols, the superior threshold is obtained starting at 90% of the MSO and decreasing by 10% steps until less than 10 MEPs out of 10 TMS stimulus are obtained and then increasing by 1% steps until 10 MEPs out of 10 stimuli are

present again). The MT is then calculated by taking the arithmetic mean of the lower and the upper motor thresholds (Groppa et al., 2012, Mills and Nithi, 1997).

3. Adaptive methods

In contrast with the aforementioned protocols based on frequency estimation, the adaptive methods adopt a probabilistic approach that calculates the probability of evoking an MEP at a given stimulus intensity.

3.1. Awiszus' threshold-hunting algorithm

This method utilises mathematical programs to calculate the most likely MT intensity based on the results (success or failure to elicit an MEP) of previous stimulus intensities. Awiszus incorporated a tool assessment called Motor Threshold Assessment Tool (MTAT), which uses the maximum-likelihood strategy for estimating motor thresholds. This method is a modification of the best Parameter Estimation by Sequential Testing (PEST) method, an adaptive method based on PEST and Maximum Likelihood (ML) regression (Pentland, 1980). Awiszus does not use prior knowledge of MT. The maximum likelihood regression algorithm program is freely available by Awiszus and Borkardt. TMS Motor Threshold Assessment Tool (MTAT 2.0: htpp://www.clinicalresearcher.org/software), 2011.

The best PEST method estimates the MT by fitting the data in an S-shaped logistic function that models the relationship between the probability of eliciting an MEP at a given TMS intensity. The function resembles a cumulative Gaussian model as a monotonical increase of the probability to elicit an MEP is seen with increasing stimulus intensities. The data is obtained by stimulating at variable intensities and applying the maximum likelihood regression algorithm. The pulse is delivered, and then the outcome of this particular intensity (success or failure to evoke an MEP) is

entered into the program. A new intensity for the next TMS pulse is estimated as the intensity with a 50% probability of generating an MEP, based on all available data and the ML regression algorithm. The procedure continues until two consecutive intensity values with the same MT prediction are found.

3.2. Bayesian Adaptive Method

This method applies Bayesian regression for PEST. The advantage over the best PEST method is to incorporate prior MT knowledge systematically. This means that after each pulse, the likelihood of MEP generation by the model is combined with the prior Bayesian regression distribution of the MT at a group level or subject-specific level. This generates a posterior probability distribution of MT to determine the intensity of the next pulse. Subsequent trials will add information input to the set, and as consecutive regressions are performed with the new data, the MT distribution probability will be updated until the probability stopping criteria are met.

Compared with the best PEST method, the Bayesian method requires fewer pulses for MT calculation, and the final MT values obtained by this protocol do not show much difference when compared with other adaptative methods (Qi et al., 2011).

In conclusion, the IFCN supports the adaptative methods based on threshold tracking algorithms as a preferable option over relative frequency methods (Groppa et al., 2012). The adaptative protocols offer the advantage of being more time-efficient by requiring fewer pulses than the frequency methods or providing a more accurate estimation with the same number of pulses (Awiszus, 2011, Qi et al., 2011).

Cortical silent period (CSP)

The CSP is the transient interruption of the volitional electromyographic (EMG) activity following TMS stimulation in the contralateral hemisphere. Inhibitory spinal processes contribute to the very early CSP (<50ms), being the latest part generated by motor cortical inhibition.

The spinal mechanisms involved in the CSP include the activation by descending cortical motor fibres of spinal inhibitory interneurons (Ia) and the inhibition of spinal motor neurons by Ib afferent fibres after muscle contraction. However, this spinal inhibition is limited to the first 50 milliseconds of the CSP (Person and Kozhina, 1978, Ziemann et al., 1993).

The CSP follows the MEP after suprathreshold TMS stimuli. However, the physiological CSP threshold is lower than the MEP threshold, and a low-intensity TMS would induce CSP in the absence of a preceding motor response. This supports the cortical origin of the CSP in the absence of spinal excitation. Therefore, CSP reflects, for the most, motor cortical inhibition (Davey et al., 1994). Epidural recordings showing the inhibition of the late indirect waves, when two TMS pulses were delivered 100-200 milliseconds apart, further support cortical inhibition as the major contributor to the CSP (Chen et al., 1999).

The CSP is believed to be related to the inhibitory effect of the gamma-aminobutyric acid B receptors ($GABA_BR$) in the motor cortex (Ziemann, 2005, Ziemann, 2004). However, many other physiological and biochemical mechanisms, such as dopaminergic systems between others, influence the CSP (Kimiskidis et al., 2006).

Overall, the MT and CSP have different underlying physiological mechanisms reflecting the excitability of the corticospinal pathway and the strength of the cortical inhibition, respectively (Ozyurt et al., 2019, Ziemann et al., 1996).

C.1.3.2.3. Paired pulse TMS (ppTMS) parameters: Intracortical facilitation (ICF), Intracortical inhibition (ICI)

Paired pulse TMS (ppTMS) stimulation assesses cortical circuits. The cortical origin of the inhibitory and facilitatory phenomena measured by the ppTMS protocols has been elegantly demonstrated by experimental epidural recordings of the corticospinal tracts (Di Lazzaro et al., 1999, Di Lazzaro et al., 1998, Di Lazzaro et al., 2008, Di Lazzaro et al., 2012). A conditioning TMS stimulus (CS) applied before a test stimulus (TS) probes inhibitory or excitatory changes in cortical excitably. The CS intensity may be delivered either above or below the threshold for eliciting MEPs, and different interstimulus intervals (ISIs) between the CS and the TS can be applied. This allows the exam of the following intracortical inhibitory and facilitatory mechanisms:

1. Short interval intracortical inhibition (SICI). A subthreshold CS preceding the suprathreshold TS by 1-5 milliseconds results in a decrement in the MEPs' amplitude. This inhibition of the TMS MEPs is believed to be a gamma-aminobutyric acid A receptors ($GABA_AR$) mediated inhibitory process (Kujirai et al., 1993). The subthreshold CS is believed to activate low threshold inhibitory interneurons in the motor cortex, causing a suppression of the late I waves (see section 1.3.1.5). The I1 wave is not modified (Di Lazzaro et al., 2018). This suggests that the SICI protocol does not interfere with the response of the pyramidal neurons to the excitatory inputs. The SICI paradigm does enhance the GABAergic inhibitory inputs probing the

excitability of the inhibitory interneuronal circuits in the motor cortex (Di Lazzaro et al., 2012).

Other authors support two distinct phases in the SICI. An early phase at one millisecond ISI and a later phase at 3-5 millisecond ISI. Both phases are generated at a cortical level by GABAergic inhibitory synaptic mechanisms. In the early phase, a superadded axonal relative refractory period in a small number of pyramidal tract neurons overlaps the synaptic mechanisms. This is justified by the suppression of I1 and the partial suppression of magnetic D waves in addition to the late I 'waves' suppression (Fisher et al., 2002, Hanajima et al., 2003).

2. Intracortical Facilitation (ICF). A protocol with a longer ISI where a subthreshold CS precedes the TS by 6-25 milliseconds would increase the size of the MEP. This facilitation is believed to be a glutaminergic mediated excitatory process (Chen, 2004, Ziemann et al., 1998a). Increased ICF may be a consequence of increased glutaminergic activity or failure of $GABA_A$ modulation over the excitatory circuits (Fedi et al., 2008, Badawy et al., 2009a).

3. Short interval intracortical facilitation (SICF). Intracortical facilitation is observed at discrete short-interval ISIs either at 1.1-1.5 milliseconds, at 2.3-3.0 milliseconds, and at 4.1-5.0 milliseconds. Conversely to the aforementioned ppTMS paradigms, a suprathreshold or at threshold CS is followed by a below threshold TS that provokes facilitation of the MEP. This phenomenon is believed to be of cortical origin (Ziemann et al., 1998b) through the facilitation of neural circuits involved in late I-waves generation. The suprathreshold CS generates excitatory postsynaptic potentials in the excitatory cortical interneurons, making them hyperexcitable. The axons of these

neurons are subsequently activated by the test stimulus resulting in a late I-wave mediated facilitation (Ilic et al., 2002).

4. Long interval intracortical inhibition (LICI). Longer ISI_S with a suprathreshold CS preceding suprathreshold TS by 100-200 milliseconds result in intracortical inhibition, which is believed to be a gamma-aminobutyric acid B receptor ($GABA_BR$) mediated inhibitory process (Valls-Sole et al., 1992, Di Lazzaro et al., 2018)

C.1.3.3. TMS-EEG parameters

TMS-EEG is the incorporation of brain stimulation by TMS and simultaneous EEG recordings. This has become achievable due to technical improvements allowing TMS coupling to the EEG. The TMS-EEG allows the assessment of both the local and distant effects of TMS, making this technique a valuable tool in the study of both cortical excitability (local) and connectivity (propagation of TMS evoked activity). Overall, the TMS-EEG combination provides information on cortical reactivity in real-time, opening new avenues for neurophysiology research in the dynamics and hierarchical organisation of brain functions (Miniussi and Thut, 2010).

C.1.3.3.1. Physiological mechanisms underlying TMS-EEG parameters

TMS-EEG utilises the EEG to record the output of the brain response to the TMS pulse. After TMS stimulation, periods of synchronised brain activity can be seen after the pulse. The TMS induced electric currents depolarise the neuronal cell membranes and open the voltage-gated ion channels generating action potentials. This would result in synaptic activation, postsynaptic potentials and extracellular current flow recorded in the EEG. The response to TMS is recorded from all the areas of the brain with the EEG and both the spatial and temporal aspects of the TMS (Ilmoniemi et al., 1997), reflecting local excitability and functional connectivity (Komssi et al., 2002,

Komssi et al., 2004, Komssi et al., 2007, Massimini et al., 2005, Ferreri et al., 2011) are evaluated.

One single pulse at an adequate intensity causes changes in the EEG lasting for at least 300 milliseconds, and these changes are not topographically limited to the area directly stimulated. The very early responses occur near the stimulation area, but subsequently, the responses propagate to different brain regions (Rogasch et al., 2014a, Rogasch et al., 2014b). The initial TMS-evoked response seems to be caused by the local activation of the stimulated area, and the later responses are due to axonal propagation of the signals. The transmission of the signals is influenced by the functional stage and degree of local activation at the time of stimulation (Ilmoniemi and Kicic, 2010, Veniero et al., 2013) and by the state of the neural network (Kahkonen et al., 2001, Massimini et al., 2005).

C.1.3.3.2. TMS-EEG responses: standard TMS-evoked potentials (TEPs), oscillatory activity, event-related synchronisation-desynchronisation

Besides the standard TMS evoked potentials (TEP), TMS also elicits event-related EEG synchronisation/desynchronisation (Rossini et al., 2015) through modulation of oscillatory activity or temporary disruption of the ongoing physiological rhythms (Paus et al., 2001, Fuggetta et al., 2005, Van Der Werf and Paus, 2006, Van Der Werf et al., 2006, Rosanova et al., 2009, Maki and Ilmoniemi, 2010a, Veniero et al., 2011, Thut et al., 2011, Vernet et al., 2013, Garcia Dominguez et al., 2014, Shafi et al., 2014, Pfurtscheller and Lopes da Silva, 1999)

The TEPs are the average response across trials in the time domain of all the scalp recording electrodes to study TMS evoked activity that is highly time-locked with the TMS pulse, and they can be visualised as topographic plots mapping each of the

multiple scalp EEG channel's TEPs. The TEPs amplitude is typically the highest under the TMS coil and diminishes with increasing distances from the initial stimulation areas. This reflects a TMS local effect (local excitability) and a TMS distant effect due to the transmission of the TMS-evoked activity through the cortical networks (Scherg, 1992, Hamalainen and Ilmoniemi, 1994, Ilmoniemi et al., 1997, Paus et al., 2001, Ferreri et al., 2011).

The event-related synchronisation-desynchronisation analysis studies the brain response to TMS in the frequency domain, assessing how the TMS stimulus modulates ongoing oscillatory rhythms. The averaged evoked response can be transformed into a time-frequency domain to assess oscillations that are time-locked to TMS pulses (evoked oscillations) (Pellicciari et al., 2017, Rogasch et al., 2014b). The activity in the different frequency bands directly evoked by the TMS pulse can be measured as a transitory phase alignment of the oscillatory activity (Thut et al., 2011, Kawasaki et al., 2014).

TMS is believed to influence cortical oscillations at both local and remote locations. The evoked oscillatory response to the TMS pulse varies in the different brain regions. Specifically, the oscillatory response to stimulation over the occipital regions is in the alpha and gamma range (Rosanova et al., 2009, Pigorini et al., 2011, Herring et al., 2015), over parietal regions in the beta range (Rosanova et al., 2009), over the premotor cortex in the beta and gamma range (Canali et al., 2017) and over the frontal regions oscillations in the alpha (Pigorini et al., 2011), fast beta and gamma bands (Rosanova et al., 2009) range.

C.1.3.3.3. Standard TMS-evoked potentials (TEPs) components

The TMS evoked EEG averaged responses, when delivered in a controlled and precise fashion, offer the advantage of a high reproducibility in contrast to the variability of the TMS motor evoked potentials (Lioumis et al., 2009, Ilmoniemi and Kicic, 2010, Casarotto et al., 2010).

The evoked EEG response to single-pulse TMS in the motor cortex shows the following components (Figure 1.5): N15 (18), P30, N45 (44), P55 (60), N100, P180 and N280. The names of the components depict their polarities and latencies. N and P letters refer to negative and positive deflections, respectively. The numbers refer to the approximate values of the peak latencies after TMS stimulus (Komssi et al., 2002, Komssi et al., 2004, Paus et al., 2001, Bender et al., 2005, Massimini et al., 2005, Esser et al., 2006, Ilmoniemi and Kicic, 2010, Komssi and Kahkonen, 2006). The N15 is the initial response seen in the electrodes placed over the stimulated motor area. The potentials propagate over central (P30) and contralateral regions (N45), with the later TEPs, N100 and P180, having a bilateral central and centro-frontal distribution (Nikulin et al., 2003, Bonato et al., 2006, Kicic et al., 2008).

The P30, N45 and N100 are the more robust responses (Lioumis et al., 2009). Very early responses, N7 and P13, have been reported in some studies (Ferreri and Rossini, 2013), but these potentials may be difficult to disentangle from the scalp muscle activity elicited by TMS (Mutanen et al., 2013, Rogasch et al., 2013b).

Other works have also described the TEPs evoked by TMS stimulation over the dorsolateral prefrontal cortex (DLPFC). These TEPs are P25, N40, P60, N100 and P185. When compared to the potentials evoked by stimulation in the motor area, they have smaller amplitudes, and the latencies also differ (Kahkonen et al., 2005b). As in

the case of the motor TEPs, the initial components also occur in the electrodes over the stimulated area with further propagation of the later components over contralateral frontal (P60) and bilateral central areas (N100, P180). The N100 and P180 are the most robust and reliable components (Kerwin et al., 2018). TEPs evoked by stimulation in the premotor cortex, supplementary motor area, posterior parietal cortex, and occipital cortex have been described, but their components are not well characterised (Tremblay et al., 2019).

C.1.3.3.4. Physiological mechanisms involved in the TEPs' components generation

Each of the TEPs components represents the sum of the pyramidal neurons' excitatory and inhibitory postsynaptic potentials (Rogasch and Fitzgerald, 2013).

The early motor TEPs peaks (N15–P30) probably convey local cortical excitability (Hill et al., 2016, Maki and Ilmoniemi, 2010b), while the later peaks (N45–N100) are believed to manifest cortical inhibition.

The slow excitatory postsynaptic potentials (EPSPs) mediated by N-methyl-D-aspartate (NMDA) receptors activated by glutamate are believed to be involved in the generation and/or modulation of the N7 component (Ferreri et al., 2011). The inhibitory postsynaptic potentials (IPSPs) mediated by $GABA_A$ receptors are probably associated with the generation and/ or modulation of N18, P30 and N45 components (Premoli et al., 2014). Also, $GABA_B$ mediated inhibitory processes are proposed to be involved in the P55, N100, P180 and N280 generation (Rossini et al., 2015, Premoli et al., 2014, Rogasch et al., 2013a, Hill et al., 2016).

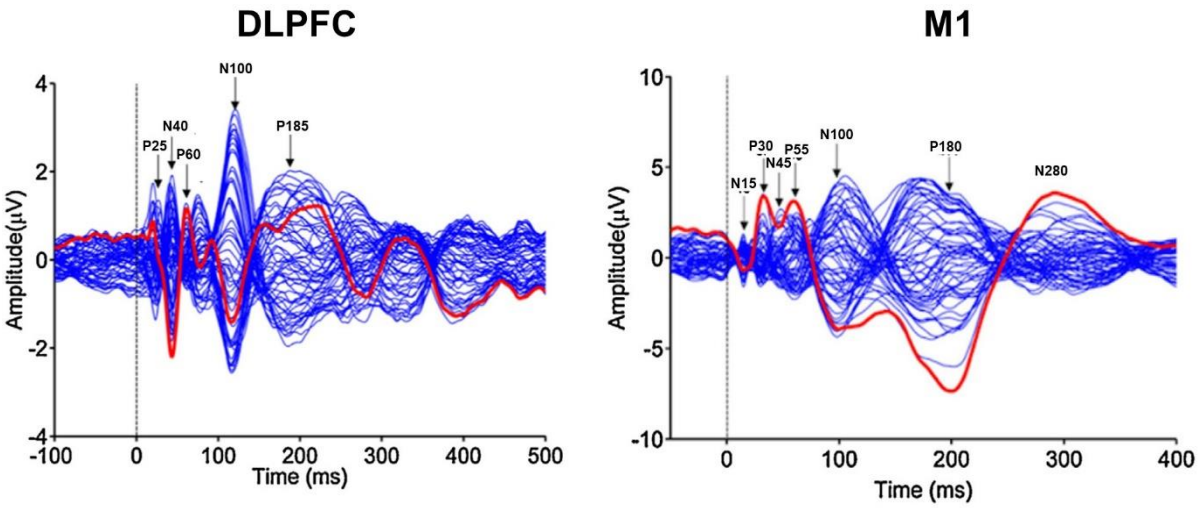


Figure 1.5. Butterfly plot representation of the EEG evoked potentials obtained upon the average of EEG epochs and single-pulse TMS stimulations of the motor cortex (right) and dorsolateral prefrontal cortex (left). The principal components evoked by motor cortex TMS stimulation (N15, P30, N45, P55, N100 and P180) and dorsolateral prefrontal cortex TMS stimulation (P25, N40, P60, N100 and P185) are displayed in the figure. Motor (M1), dorsolateral prefrontal cortex (DLPFC). Modified from Rogasch et al. and Hills et al.

C.1.3.3.5. Quantification of TEPs

The TEPs are recorded from electrodes located in regions both close and distant to the TMS stimulated area (Ilmoniemi et al., 1997, Massimini et al., 2005, Rosanova et al., 2009)**.** The TEPs can be assessed locally and globally. To quantify local TEPs from a region of interest (ROI), EEG signals from neighbouring electrodes are averaged, and the amplitude and latency of the TEP components are measured. TEPs are graphically represented as a waveform of fluctuating amplitude and changing polarity as a function of time (Figure 1.5). TEPs can also be represented as voltage maps to visualise at selected time points the spread of the potential across the cortex from the area of stimulation (Hill et al., 2016). The local mean-field power (LMFP)

(Pellicciari et al., 2013, Romero Lauro et al., 2014) is another method to study the TEPs. LMFP measures the area under the curve of the rectified signal of the electrodes of interest. The curve is computed as the standard deviation across channels (root mean square) at a given point in time. The advantage of LMFP over the amplitudes/ latency measurements is that it also considers the duration of the component, and it does not require a clear peak for accurate measurement. However, as the signal is rectified, the polarity of the signal is ignored.

In order to depict global TEPs across the entire brain, the global mean-field potential (GMFP) is utilised. The GMFP display the effect of the TMS pulse in the activity across all the recording electrodes as the averaged signal of TMS activity over the entire surface of the scalp. GMFP corresponds to the standard deviation (root mean square) across all electrodes at a given point in time (Lehmann and Skrandies, 1980, Komssi et al., 2004, Esser et al., 2006). GMFP and LMFP integrate all the TEP data at a local or global level, respectively and facilitate the comparison of multiple stimulation sites (Kahkonen et al., 2005a, Fecchio et al., 2017).

A different analytical approach for comparison of all the TEP data points across space and time can be obtained through statistical methods like the nonparametric, cluster-based permutation tests (Garcia Dominguez et al., 2014, Premoli et al., 2014, Rogasch et al., 2014a). These methods allow for a mass-univariate analysis of the TEPs parameters such as amplitude and frequency oscillations across time and space while correcting the multiple comparisons problem (Maris and Oostenveld, 2007).

Similar to TMS-EMG, TEPs can be evoked by paired-pulse protocols in which two pulses (conditioning and test pulses) are delivered at certain inter-stimulus intervals

(ISI). The differences between the TEP component obtained with or without previous conditioning are compared**.**

C.1.3.3.6. Measurement of TMS-related cortical oscillations

The most common methodology used to analyse the TMS-related cortical oscillations is based on measuring the power of these oscillations across time and frequency with signal processing techniques such as wavelet decomposition and short-time Fourier transforms (Farzan et al., 2016, Pellicciari et al., 2017).

There are two different approaches for the assessment of the TMS related oscillations. For the analysis of TMS time-locked oscillations (evoked oscillatory responses-EOR), the time-frequency decomposition analysis is applied to the data averaged across trials. On the other hand, if the time-frequency decomposition is applied to single trials, all the oscillations, including those not necessarily time-lock to the pulse (called induced oscillations), are included in the analysis. These total oscillatory responses (TOR) are also known as event-related spectral perturbations (ERSPs) and refer to all time-locked and non-time locked TMS related oscillations (Herrmann et al., 2014).

C.1.3.3.7. TEPs variability and reproducibility

The TEPs elicited by single-pulse stimulation over the motor area are subjected to idiosyncratic interindividual differences, but the TEPs are highly reproducible in the same subject across time, if the same stimulation parameters are used (Casarotto et al., 2010).

However, the TEPs are very responsive to modifications in the TMS parameters. The responses can markedly change in response to changes in the stimulus intensity (Komssi et al., 2004, Kahkonen et al., 2005b, Casarotto et al., 2010), changes in the coil orientation (Bonato et al., 2006, Casarotto et al., 2010) and position (Komssi et

al., 2002). Stimulation over different cortical regions evoked distinct TEPs outputs (Kahkonen et al., 2004, Kahkonen et al., 2005a, Fitzgerald et al., 2008, Rosanova et al., 2009).

TEPs waveforms are also modified by the level of alertness, vigil or different sleep stages (Massimini et al., 2005, Massimini et al., 2012) and the attentional state of the cortex, e.g. preparation to initiate a movement or engagement in a task (Nikulin et al., 2003, Ferreri et al., 2014, Kundu et al., 2014).

The brain state and engagement in attentional tasks also have an impact on the oscillatory responses to TMS, e.g., when attending to a visual stimulus, the TMS stimulation of the occipital region evoked a more lasting alpha oscillation than when attending to an auditory stimulus (Herring et al., 2015).

C.1.3.3.8. Reasons and advantages of the TMS-EEG technique

TMS-EEG technique offers several benefits over TMS-EMG for the study of cortical excitability. First, TMS-EEG responses can be elicited at intensities below motor threshold (40% of the MEP threshold) (Komssi et al., 2004, Komssi and Kahkonen, 2006, Komssi et al., 2007). Furthermore, TEPs are recorded from both local and distal electrodes to study the propagation of the evoked activity across cortical regions (Ilmoniemi et al., 1997, Komssi et al., 2002). Therefore, in the same way that the TMS-EMG provides an indirect measure of motor cortical excitability through EMG recordings, the TMS-EEG quantifies neurophysiological parameters in the motor and non-motor cortical areas through TEPs. Thus, while TMS-EMG assessments are limited to the boundaries of the cortico-spinal motor pathways, the evoked TMS-EEG responses allow the study of excitability in cortico-cortical and cortico-thalamic circuitry. Alterations in the induced oscillatory rhythms generated by TMS appear to

be associated with diverse neuropsychiatric conditions, as the example described by the Ferrarelli group (Ferrarelli et al., 2008).

The TMS-EEG offers new possibilities for the investigation of the physiopathology of the Central Nervous System (CNS) as a non-invasive stimulation technique offering a number of advantages and scope for further applications:

- Identification of task correlated cortical activity in areas previously localised by functional neuroimaging studies (fMRI) (Sack, 2006).
- EEG recording and mapping of the TMS induced neuronal responses (Ilmoniemi and Kicic, 2010, Komssi and Kahkonen, 2006, Ilmoniemi et al., 1997).
- Study of the functional connectivity between brain areas in health (Miniussi and Thut, 2010, Miniussi et al., 2010) and disease (Kugiumtzis and Kimiskidis, 2015).
- Evaluation of the impact of the TMS stimuli on oscillatory brain activity (Paus et al., 2001).
- Assessment of changes in neuronal activity or excitability (TMS-aftereffects) during and after different TMS stimulation protocols; for review, see (Thut and Pascual-Leone, 2010).
- Gain insights into the functional dynamics of cortical networks by analysing TEPs responses over the entire scalp (Kahkonen et al., 2005b, Miniussi and Thut, 2010)
- Study the chronometry of intra- and inter-hemispheric transmission of TMS-induced potentials. This study, combined with modelling of neuronal generators, aids in the investigation of causality in neuronal networks (Komssi et al., 2002).

- Expand the knowledge about the cortico-cortical and interhemispheric interactions (Bonnard et al., 2009) by stimulating a selected target with TMS and recording the responses at distant interconnected areas (Mochizuki et al., 2004, Silvanto et al., 2006).
- Evaluation of the changes in the general state of the brain under certain interventions, e.g., the study of the modulatory effects of exogenous substances in cortical activity (Kahkonen et al., 2001) or conditions, e.g., reduced functional connectivity of brain areas during non-REM sleep has been described (Massimini et al., 2005).
- Study of the interaction between areas of the brain during sensory processing (Bikmullina et al., 2009, Raij et al., 2008, Silvanto et al., 2006) or during the control of a motor task (Nikulin et al., 2003, Kicic et al., 2008, Mochizuki et al., 2004).
- Implementation of TEPs as a prospective tool for the assessment of the effectiveness of repetitive TMS (rTMS) to modulate cortical activity (Casula et al., 2014, Esser et al., 2006, Helfrich et al., 2012, Vernet et al., 2013).
- Improve the understanding of physiological human brain functions, for instance, the Long-term Potentiation (LTP). Esser et al. studied the phenomenon of LTP following rTMS measuring the global mean field power (GMFP). The study demonstrates increasing amplitude of the evoked responses to a single pulse TMS in areas distant to the stimulated zone due to activation of excitatory pathways following an rTMS session (Esser et al., 2006).
- Safety control during the implementation of repetitive TMS as a treatment choice for drug-resistant epilepsies. TMS-EEG may assess the effects of rTMS during and after a therapeutic session (Kimiskidis, 2016). TMS-EEG monitors

the presence of provoked epileptiform discharges (EDs) during the rTMS. This would assist in the decision-making regarding optimization of the rTMS stimulation parameters or the interruption of the session, for review see (Rotenberg, 2010, Kimiskidis, 2010).

- Measure the cortical excitability at baseline or after antiepileptic treatment (Rotenberg, 2010).

The applications of TMS-EEG in the diagnosis of epilepsy will be explained in detail in chapter C.1.4. TMS and Epilepsy (section, C.1.4.3.)

C.1.4. Transcranial magnetic stimulation (TMS) for the diagnosis of epilepsy

C.1.4.1. TMS and epilepsy general introduction

TMS provides safe, non-invasive and sensitive measurements of neuronal excitability, assessing the excitatory and inhibitory functions of cortical neurons (Macdonell et al., 2002). TMS evolved from a technique limited to the study of motor pathways to an innovative tool in the field of neurosciences with diverse research, diagnostic and therapeutic applications (Macdonell et al., 2002, Tassinari et al., 2003, Theodore, 2003, Kimiskidis, 2010). In particular, TMS may assist as an indicator of pathological excitatory-inhibitory cortical imbalance in certain conditions. TMS has the potential to be a biomarker of epilepsy (in the sense of assessing a measurable functional change that denotes the presence of a particular disease), and it can localise areas of cortical hyperexcitability in focal epilepsies (Valentin et al., 2008).

C.1.4.2. Transcranial magnetic stimulation and electromyography (TMS-EMG) parameters as a diagnostic tool in epilepsy

Traditionally combined with electromyography, TMS has been implemented by several groups to assess changes in motor cortical excitability in epilepsy patients (Reutens and Berkovic, 1992, Manganotti et al., 2000, Werhahn et al., 2000, Hamer et al., 2005, Badawy et al., 2007). TMS-EMG studies in patients with epilepsy have shown conflicting results. For instance, studies using small cohorts of drug-naive patients showed varying results (Reutens et al., 1993, Delvaux et al., 2001, Werhahn et al., 2000, Varrasi et al., 2004, Brodtmann et al., 1999). Furthermore, a number of studies have been conducted on patients with long-standing epilepsy treated with antiepileptic drugs (AEDs). As the AEDs interfere with various TMS-EMG parameters used to

measure cortical excitability, it was difficult to disentangle the drug-induced changes from those exclusively due to epilepsy (Ziemann et al., 1996). TMS-EMG has demonstrated a decreased MT in untreated patients with genetic generalised epilepsy compared to healthy controls (Reutens et al., 1993). On the other hand, studies in progressive myoclonic epilepsy did not show decreased MT values but revealed impaired cortical inhibition (Reutens et al., 1993, Valzania et al., 1999, Manganotti et al., 2001). In one of these studies, a loss of cortical inhibition and an increase in facilitation was reported with paired-pulse paradigms at 100–150 milliseconds and 50 milliseconds intervals, respectively (Valzania et al., 1999).

The applications of TMS-EMG in epilepsy have been extensively reviewed (Macdonell et al., 2002, Tassinari et al., 2003) and can be broadly classified into the following groups:

- In-vitro and in-vivo experimental studies investigating the parametrisation and neurobiological interpretation of TMS effects in humans (Vahabzadeh-Hagh et al., 2012).
- Application of single-pulse and paired-pulse TMS protocols as a diagnostic procedure for measurement of cortical excitability (de Goede et al., 2016).
- Non-invasive investigation of cortical excitability in patients with epilepsy. Changes in cortical excitability, measured as an index of intra-cortical inhibition/ facilitation, have been associated with the pre-or post-ictal phase (Badawy et al., 2009a). These changes may even play a clinical role in the short-term prediction of seizure reoccurrence (Wright et al., 2006, Badawy et al., 2009a).
- Investigation of the mechanisms related to specific epilepsy syndromes. TMS-EMG studies in a large population of drug-naive patients with newly diagnosed GGE and in focal epilepsies with origin outside the motor cortex have

investigated cortical excitability and inhibition. The disturbance of TMS-EMG cortical excitatory/ inhibitory functions was bilateral in GGE, while in focal epilepsy remained lateralised to the affected hemisphere (although spreading beyond the actual focus) (Badawy et al., 2007). This study suggests a reduction in both $GABA_A$ and $GABA_B$ mediated cortical inhibition in epilepsy. This reduction is bilateral and diffuse in GGE and ipsilateral in focal epilepsy, supporting differences in cortical pathophysiology between the two types of epilepsy.

- Determination of the AEDs' effects on the human brain functions. AEDs treatment aims to correct the increased cortical excitability characteristically occurring in epilepsy (McCormick and Contreras, 2001). A measure of the effect of AEDs on cortical excitability and inhibition may provide insightful information to guide treatment choice. The AEDs have different modes of action and affect several TMS parameters used to assess cortical excitability (Ziemann et al., 1996, Boroojerdi, 2002). It has been established in the literature that voltage-gated sodium and calcium channel blockers elevate the corticomotor threshold, while GABAergic AEDs increase intra-cortical inhibition and reduce intra-cortical facilitation (Tassinari et al., 2003, Kazis et al., 2006). Specific effects of various anticonvulsants in TMS-EMG parameters were studied in normal subjects, showing increased intracortical inhibition with GABAergic AEDs such as Vigabatrin and increased motor threshold with sodium and calcium channels blockers such as Carbamazepine, Lamotrigine, and Phenytoin (Ziemann, 2004). TMS-EMG studies have also investigated the effects of prolonged AEDs use (Lee et al., 2005, Cantello et al., 2006, Kazis et al., 2006, Turazzini et al.,

2004, Manganotti et al., 1999), the acute effects of AEDs in healthy individuals (Ziemann, 2004), and the effects of AEDs withdrawal (Wright et al., 2006).

- Quantification of physiological effects of the AEDs in individual patients. The role of TMS-EMG in the measurement of AEDs effects in individual patients may prove to be more informative than AED blood levels. For instance, evidence in the literature has suggested that TMS could help to predict seizure control. Badawy's group studied the effect of AEDs in a cohort of patients with newly diagnosed epilepsy. The study implemented TMS-EMG measures of cortical excitability using paired-pulse stimulation at short and long interstimulus intervals (ISIs). One year of seizure freedom was marked by the evident effect of the AEDs in the TMS-EMG parameters soon after starting the treatment. Particularly the intracortical inhibition at long ISIs (250 milliseconds) was maximally increased after treatment in the respondent group. The decrease of cortical excitability in the respondent group was reported independently of the seizure type, and the AED used. A failure to show appropriate changes in TMS-EMG measures of cortical excitability after AED introduction may be a useful early predictor of pharmacoresistance in individual patients (Badawy et al., 2010, Badawy et al., 2013a).
- Non-invasive mapping of the eloquent primary motor cortex during presurgical evaluation of epileptic patients combining TMS-EMG with non-invasive neuro-navigation techniques (Sondergaard et al., 2021).

The aforementioned literature clearly highlights the major relevance of TMS for epilepsy research and clinical management. All these studies have the disadvantage of using the TMS-EMG technique, stimulating the motor cortex and recording the

evoked responses exclusively from muscles rather than the cortex itself. Stimulation across the entire cortex, unrestricted to the motor areas, and recordings of the cortical responses per se would be a more appropriate approach to investigate mainly cortical disease processes operating in epilepsy. Therefore, it is logical to assume that the combination of the EEG and TMS (TMS-EEG) would be a relevant technique to investigate pathophysiological aspects of epilepsy and, in the clinical arena, to increase the diagnostic and prognostic power of the conventional EEG.

C.1.4.3. Transcranial magnetic stimulation and electroencephalography (TMS-EEG) parameters as a diagnostic tool in epilepsy

TMS-EEG consists of the simultaneous stimulation of the brain with brief magnetic pulses through the scalp while recording the EEG responses. The integration of TMS brain stimulation with simultaneous EEG recording has become possible due to the technical developments that allowed TMS coupling to real-time EEG. The TMS-EEG combination provides immediate information on cortical reactivity and connectivity (Miniussi and Thut, 2010), facilitating a new range of applications to assess cortical excitability and cortical connectivity, specifically in epilepsy (Theodore, 2003, Schrader et al., 2004, Fregni et al., 2006, Bae et al., 2007). TMS-EEG may provide a baseline measure of cortical excitability to assess responsiveness to AEDs treatment and also may be of use as an activation technique to provoke epileptiform activity during EEG recordings (Rotenberg, 2010).

C.1.4.3.1. Why use combined transcranial magnetic stimulation and simultaneous EEG (TMS-EEG) for the diagnosis of epilepsy?

The EEG is a record of cortical electrical activity that can show a number of abnormalities in epilepsy, even during periods with no seizures (interictal period). The

hallmark of epilepsy is excessive cortical excitability (or decreased inhibition) that results in excessive bursts of neuronal activity, manifesting as the interictal epileptiform discharge (IED). IEDs are the most common abnormality seen in the EEG of patients with epilepsy. A number of activation procedures are routinely used during EEG recording to increase the likelihood of recording IEDs (hyperventilation, visual stimulation with flashing lights, sleep). However, even with the use of such activation procedures, the sensitivity of the EEG in epilepsy is relatively low (see C.1.2.4.1). An obvious approach to the study of cortical excitability would be to stimulate the cortex with short pulses of electrical currents and to record cortical responses to stimulation. Such an approach has been very useful in patients with intracranial electrodes (Valentin et al., 2002, Valentin et al., 2005b, Valentin et al., 2005a, Enatsu and Mikuni, 2016, Enatsu and Mikuni, 2018, Matsumoto et al., 2017, Zhang et al., 2018, Zhao et al., 2019, Donos et al., 2016). Unfortunately, cortical electrical stimulation with high-intensity pulses through the skin is painful and cannot be routinely carried out. However, TMS is able to directly stimulate the cortex through the skin and brain coverings, and the simultaneously recorded EEG is able to provide a non-invasive estimation of cortical excitability in epilepsy.

C.1.4.3.2. Previous experience in the TMS-EEG and epilepsy field.

TMS-EEG has been informative in several epilepsy studies in animals and humans. Experimental research in a temporal lobe epilepsy model in rats (Rotenberg et al., 2008) reported suppression of epileptic seizures and electrographic discharges with low frequency (0.5 & 0.75 Hz) repetitive TMS. This study reveals the potential of TMS-EEG as a translational method for the parameterisation of TMS to interrupt epileptiform discharges. Murine epilepsy experiments have suggested the potential of long-interval

paired-pulse TMS as a biomarker of compromised cortical inhibition (Vahabzadeh-Hagh et al., 2011).

In focal epilepsy involving clinical cases, an innovative TMS-EEG study was performed by the KCH/KCL group (Valentin et al., 2008), and the results suggested that TMS-EEG, when combined with the standard EEG in an add-on design, has higher diagnostic sensitivity and positive predictive value than the EEG alone (see more details C.1.4.3.3).

Also, Kimiskidis et al. investigated the acute therapeutic effects of TMS in patients with focal epilepsy, confirming that repetitive TMS can reduce the duration of the epileptiform discharges from superficial epileptogenic foci (Kimiskidis et al., 2013). To further explain this finding, the modulatory effects of the TMS stimulation in the connectivity patterns of focal epilepsy was explored (Kugiumtzis and Kimiskidis, 2015). The study revealed a significant increase in the values of a non- linear measure of effective connectivity during electrographic seizures. This pathological feature was substantially reversed by TMS stimulation providing a plausible explanation for the acute therapeutic effects of TMS in epilepsy.

A TMS-EEG study in eight patients with periventricular nodular heterotopia (PNH) combined resting-state functional connectivity MRI (rs– fcMRI) techniques to separate "connected" regions, cortical areas with abnormal connectivity to heterotopic nodules from "non-connected" regions. In patients with active epilepsy, the single-pulse TMS stimulation of the "connected" regions resulted in an abnormal increase of the normalised GMFP in the late TEPs components (225–700 ms), suggesting cortical hyperexcitability (Pascual-Leone et al., 2011, Shafi et al., 2015) in contrast with the "non-connected" regions.

Several studies have focused on the different characteristics of the TEPs recorded in GGE patients in comparison to the TEPs seen in healthy controls. These studies implied abnormalities in cortical reactivity in GGE, manifested in the TEP's late components (N100 and P180). Syndrome-specific TEPs changes have also been described. For instance, increased amplitude of the P30 component and reduced amplitude of the N100–P180 complex in patients with Unverricht Lundborg Disease (EPM1), a subtype of progressive myoclonic epilepsy, suggest enhanced cortical excitability and reduced cortical inhibition, respectively (Julkunen et al., 2013). Sleep-deprived TMS-EEG studies have shown a much higher enhancement in cortical excitability after sleep deprivation in patients with juvenile myoclonic epilepsy (JME) than in controls (Del Felice et al., 2011). Ter Braack's study reported abnormal cortical reactivity with larger late TEPs components (N100, P180) in epilepsy patients than in controls. However, this study has some limitations, such as the heterogeneous patient sample including both partial and generalised epilepsies and the missing inclusion of drug-naive patients, the latter adding uncertainty about the results due to the possible influences of the AEDs over the TEPs (Ter Braack et al., 2016).

Further studies probing cortical excitability in GGE with a paired-pulse TMS–EEG protocol were carried out by Kimiskidis et al. group (Kimiskidis et al., 2017). This study was designed as a multi-level data analysis with two main objectives. The first was the optimisation of TMS parameters, aiming to implement the TMS as an activation procedure to provoke IEDs in GGE. In GGE, a critical mass of brain tissue has to be stimulated for the IEDs activation to be accomplished. The experimental data proved the superior effectiveness of the circular coil over the figure-of-eight coil for inducing IEDs. The stimulus intensity (SI) was also a relevant parameter. In order to find the epileptogenic threshold (SI threshold for eliciting EDs), a protocol based on direct

cortical stimulation parameters for the localisation of the epileptogenic zone was followed (Alexopoulos et al., 2007). Thus, increasing SI were delivered to elicit IEDs while taking precautions to avoid undesirable side effects. In the second part of the study, single and paired-pulse TMS–EEG was performed at rest, during and after hyperventilation (HV) in 25 GGE patients and 12 controls. After prospective follow-up, the GGE patients were classified as AEDs responders (n=13) and non-responders (n=12). A feature selection scheme of TMS–EEG parameters and a Bayesian classifier was used to calculate the accuracy of an index test to assign the subjects into epileptic and controls and AEDs responders and non-responders' categories. The results suggested the high accuracy of TMS–EEG to separate GGE patients from controls and GGE responders to AEDS from non-responders (Kimiskidis et al., 2017).

The existing publications and pilot studies in TMS-EEG for epilepsy diagnosis include the following main indications:

- Diagnostic measures of cortical excitability: EEG scalp electrodes can record TMS-evoked potentials from any cortical region and estimate regional excitability outside the motor mantle, overcoming the anatomic limitations of TMS-EMG where only the motor cortex can be assessed (Kahkonen et al., 2005b, Kahkonen et al., 2005a) (Lioumis et al., 2009).

- Study the potential of the TMS as an activation technique to evoke epileptiform activity (Schuler et al., 1993, Kimiskidis et al., 2017).

- Non-invasive localisation of the epileptogenic zone and optimization of the EEG diagnosis yield for focal epilepsy. TMS has the potential to enhance the sensitivity of the scalp EEG to identify epileptiform abnormalities (Valentin et al., 2008, Kimiskidis et al., 2017).

C.1.4.3.3. Experience at Kings College London/ Kings' College Hospital

The previous pilot study from Kings College London/ King's College Hospital (KCL/ KCH) group strongly suggests that TMS-EEG has the potential of becoming a very useful diagnostic tool for patients with epilepsy (Valentin et al., 2008). In particular, TMS has potential as a biomarker of epilepsy, and it can localise areas of cortical hyperexcitability in focal epilepsies. In this initial study, the diagnostic value of TMS-EEG in focal epilepsy was estimated in 15 patients with known long-standing chronic focal epilepsy and 15 healthy volunteers. During the TMS-EEG brain stimulation, single TMS pulses were delivered at the standard electrode positions of the 10-20 system while simultaneously recording the EEG responses. Two types of TMS evoked EEG responses were recorded: the early responses, which corresponded to the TEPs N100 waveform, and the late responses. The late responses were further subclassified into delayed responses, which resembled interictal epileptiform discharges (recorded at a latency > 100 ms and < 1-second post-TMS stimulus) or repetitive responses (new-onset repetitive rhythmic activity, seen upon averaging of 9 to 15 EEG trials time-lock to the TMS stimuli) (Figure 1.6). Patients with chronic focal epilepsy showed EEG responses to TMS that differ from volunteers, presumably due to altered cortical excitability. Interestingly, late TMS-EEG responses never occurred in the healthy control group while they appeared in 11 out of 15 epilepsy patients (mainly with extratemporal focal epilepsy). The four patients in which the TMS did not evoke late responses had deeply located epileptogenic areas (i.e., at the insular cortex or medial temporal structures). Late TMS-EEG responses were seen in some epileptic patients with normal EEG recordings, suggesting the combined use of standard EEG and EEG-TMS may increase the diagnostic yield of EEG.

The results from this study suggest that TMS-EEG can add sensitivity to the EEG to detect focal epilepsy while maintaining high specificity. TMS-EEG aids to localize the epileptogenic cortical areas and developing or enhancing the diagnostic method in focal epilepsy. The technique was less useful in temporal lobe epilepsy, as deep structures such as the hippocampus cannot be easily stimulated from outside the head.

Overall, TMS-EEG has significant diagnostic and prognostic implications in epilepsy as the TMS stimulation may elicit abnormal responses in the EEG. In focal epilepsy, TMS-EEG studies propose the TMS-EEG as a reliable tool to better define the epileptogenic zone. In GGE, abnormal TEPs may be useful for the diagnosis of epilepsy.

These results of TMS-EEG have been very promising as they seem to improve the diagnostic sensitivity of the EEG in epilepsy. However, the value of TMS-EEG for the diagnosis of newly onset epilepsies and drug naïve patients requires further investigation. The present study is designed to confirm and expand the diagnostic value of TMS-EEG in epilepsy, particularly new-onset epilepsies.

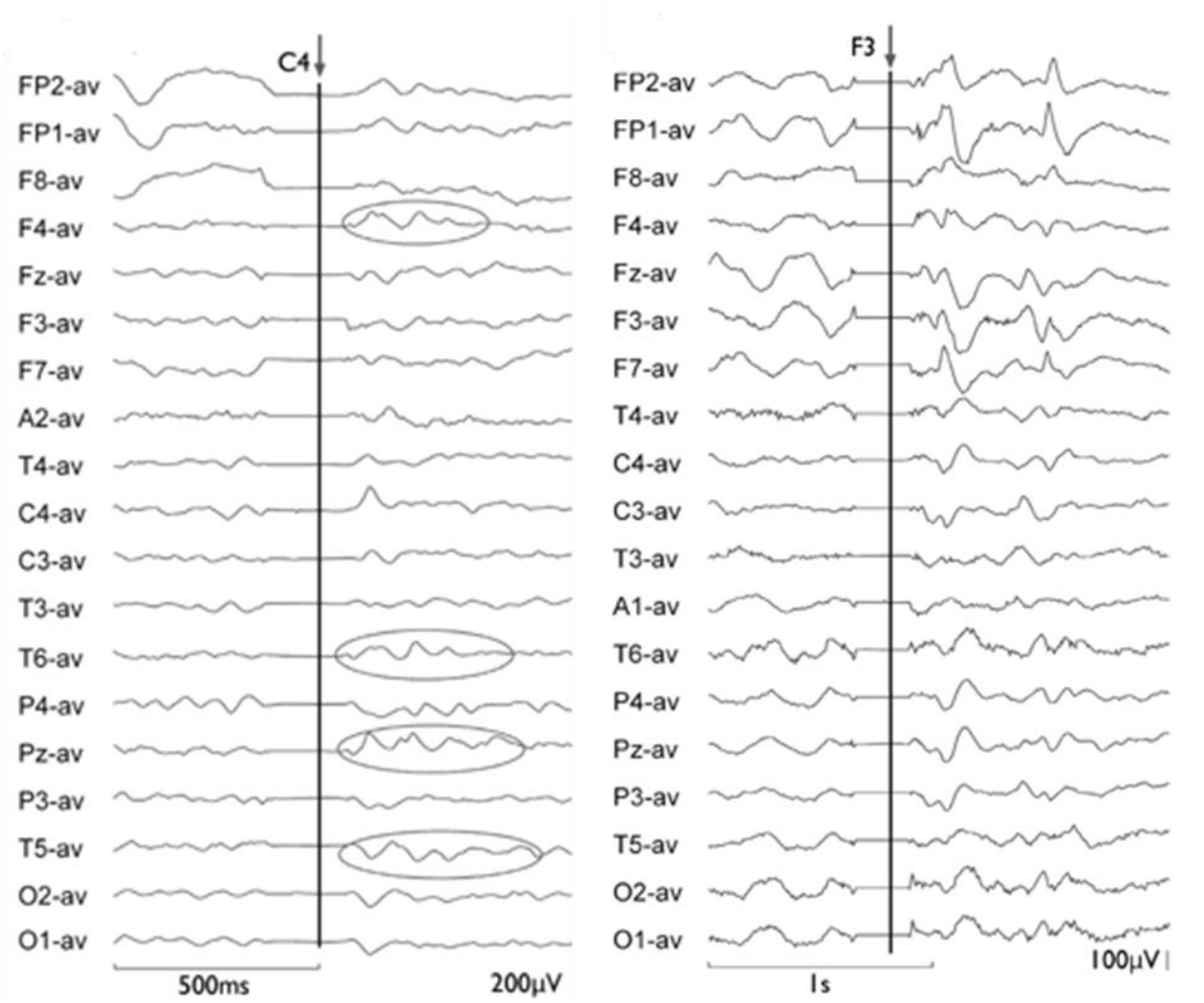


Figure 1.6. Delayed and repetitive TMS-EEG responses after TMS stimulation at C4 and F3, respectively. Left) Repetitive TMS-EEG responses are seen over F4, T6, Pz and T5 channels. Each trace is an average of nine recordings. Repetitive TMS-EEG responses have been highlighted. Right) Delayed responses over Fp2, Fp1, Fz, F3 and Fz channels. The vertical line indicates the TMS pulse. From (Valentin et al., 2008).

CHAPTER 2. MATERIAL AND METHODS

Hypothesis and aims:

The main research hypothesis is that the combined use of TMS-EEG added to the standard EEG will increase the diagnostic power of the standard EEG alone for new-onset epilepsies. Therefore, TMS-EEG could suggest a diagnosis of epilepsy or predict the development of epilepsy in patients where the first EEG is normal or inconclusive. Recent studies have suggested that the TMS-EEG responses differ when comparing subjects with true epileptic seizures, non-epileptic seizures and healthy controls (Ferrarelli, 2017, Kimiskidis et al., 2017). Therefore, the first aim of this thesis is ***to assess if the combined standard EEG and TMS-EEG confers better sensitivity than the EEG alone for earlier diagnosis of new-onset epilepsies***.

In addition, it is considered that transcranial magnetic stimulation could be able to reveal interictal excitability differences between epileptic and non-epileptics subjects, manifested as late responses to TMS and other TMS generated brain rhythms detected by automatic analysis and machine learning. Based on this concept, the second aim of this project is ***to assess the idiosyncratic characteristics of TMS induced EEG rhythms in the epileptic group as a potential biomarker of epileptogenicity***.

The third aim is ***to assess the presence or absence of quantitative features associated with epilepsy,*** such as the EEG power ratio in specific frequency bands before and after TMS stimulation.

The fourth aim is the ***selection of variables for machine learning models aiming to separate epileptic from non-epileptic subjects***.

C.2.1 Study design

C.2.1.1. Study settings

The study involves patients from the First Seizure Clinic at Guy's and St. Thomas's Hospital NHS Foundation Trust (GSTT), led by Professor Michalis Koutroumanidis. The first seizure clinic receives referrals from the Accident and Emergency department (A&E, UK term for emergency room) at GSTT, which is among the busiest A&E units in the UK. Currently, more than 150 new diagnoses of epilepsy are made in the first seizure clinic every year and classified in the various epilepsy syndromes according to the guidelines of the International league against epilepsy (ILAE) based on the history and comprehensive interictal and ictal video EEG studies (Koutroumanidis et al., 2005).

Patients attending the First Seizure Clinic at St. Thomas Hospital with suspected neurally mediated syncope will have a tilt table test and formal medical assessment in a dedicated vasovagal clinic, particularly when loss of consciousness and the associated convulsion occurs without clear preceding autonomic symptoms. Patients with suspected cardiogenic syncope are referred for full cardiological assessment with implanted loop recorders where indicated. The final diagnosis results will be considered at month 12, taking into consideration the clinical assessment (neurological, medical and cardiological, as indicated) and the appropriate diagnostic tests. However, it is expected that the diagnosis would remain uncertain in 20-30% of patients (Crompton and Berkovic, 2009).

C.2.1.2. Study participants

The specific inclusion and exclusion criteria comprise:

Inclusion criteria:

1. Adult patients reviewed in the First Seizure Clinic with a requested sleep-deprived EEG by the senior epileptologist of the clinic, Professor Koutroumanidis.
2. Patients capable of giving informed consent.
3. Patients without known TMS contraindications detected after screening with the Transcranial Magnetic Stimulation Adult Safety and Screening Questionnaire (TASS) (Keel, Smith, and Wassermann 2001))
4. Healthy volunteer adults capable of giving informed consent and without TMS contraindications.

Exclusion criteria:

1. Pregnant women.
2. Patients with pacemakers, implanted medical pumps, a metal plate in the skull, or metal objects inside the eye or inside the skull.
3. Subjects with severe neuropsychiatric or medical disorders.
4. Subjects not capable of giving informed consent.
5. Acute symptomatic seizures.
6. Patients presenting with Status Epilepticus (Rossi et al., 2009)
7. Patients not attending the follow-up consultation in the epilepsy clinic at 12 months.
8. Patients with an unclear final clinical diagnosis

C.2.1.3. Recruitment protocol

Professor Koutroumanidis selected eligible subjects, and all the procedures were fully explained to the participants at the time of their initial consultation in the First Seizure Clinic. A detailed information sheet describing the research project's aim and what this

involved was given to the patients at this stage. In order to remind the patients of the information given in the clinic and to explain in more detail the TMS-EEG system, an additional information leaflet was posted with the EEG appointment letter.

Healthy volunteers were recruited by Professor Koutroumanidis and myself from colleagues and relatives of the patients attending the First Seizure Clinic.

All potential participants were encouraged to ask questions and were given sufficient time to consider participating in the study. It was explained that participation in the study was voluntary, and they may withdraw from the study at any time without having to provide a reason. In the case of a patient, it was further emphasised that their decision to participate (or not) would have no impact on their quality of care. If the patient or volunteer agreed to take part in the study, written informed consent was obtained from each participant, and all research governance procedures were followed. A brief feedback questionnaire was completed by all the patients after the recording.

This participant's cohort is an opportunistic sample and a previous sample size calculation was not performed. However, the sample size calculations based on current evidence regarding the diagnostic accuracy of TMS–EEG (Valentin et al., 2008) estimated that a sample size of 34 participants (23 patients and 11 controls) would provide a sensitivity of 0.91 (minimum sensitivity of 0.65 and minimum specificity of 0.65), with an alpha = 0.05 and beta = 0.10 (Kimiskidis et al., 2017).

C.2.1.4. TMS-EEG settings and recordings

All participants from the First Seizure Clinic underwent a TMS-EEG evaluation on the same day as their first clinical outpatient sleep-deprived EEG study at the Department of Clinical Neurophysiology at GSTT. The sleep-deprived EEG was performed after

the initial consultation at the First Seizure Epilepsy Clinic. The participants underwent a 60 minutes standard sleep-deprived EEG recording (thereafter called baseline EEG) and were transferred from the EEG recording room to the dedicated TMS compatible EEG system area at the Department of Clinical Neurophysiology at GSTT. The baseline EEG recording was followed by a 45 to 60 minutes TMS-EEG session using notched standard round EEG electrodes that are TMS compatible, safe and well-tolerated (Valentin et al., 2008). In the case of healthy volunteers, the TMS-EEG study was not preceded by sleep deprivation or baseline EEG recording. The TMS-EEG was performed by myself, assisted by a researcher (Dr Valentin) or a clinical neurophysiologist, and we were blind to the clinical information and previous EEG results. The baseline EEG was interpreted by Professor Koutroumanidis, who was blind to the TMS-EEG data.

The hardware and software required for TMS and the TMS-EEG compatible system were a monophasic TMS stimulator (MagStim 200, serial number 10901927M) with a standard 90 mm round TMS coil. A 21 channel EEG was simultaneously recorded during the procedure with the new TMS compatible-EEG system (ASA-LAB system from ANT-BV). The ASA-LAB system has a TMS compatible 32 channels amplifier (Model ASA-ANT, Ref. 8-32e, SN 0120080042, Ref 95-0120-4006-0). Asa-Lab ANT Neuro system has a full-band EEG DC amplifier with maximum 10 kHz sampling rate. The EEG signal was digitized at a sampling frequency of 2024 Hz and continuously recorded by 21 electrodes. The 21 standard electrode positions were based on the international 10-20 system). The TMS stimulator and the EEG system functioned effectively without any disruptive EEG amplifier saturation.

A program in QuickBasic programming language was created to externally trigger the TMS stimulator while concurrently sending a specific stimulus identifier marker to the

TMS compatible EEG system. Therefore, the TMS stimulus was synchronised with a simultaneous numerical marker in the compatible TMS-EEG system allowing the identification of the TMS-EEG trials for offline analysis of the EEG responses to TMS.

In order to evaluate the motor threshold in these patients, surface EMG recordings were obtained from the right first dorsal interosseous to calculate the resting motor threshold (RMT) with the freely available Motor Threshold Assessment Tool, version 2.0 developed by Awiszus and Borckardt (htpp://www.clinicalresearcher.org/software) (Awiszus, 2003).

After the motor threshold calculation, the 21 channel TMS-EEG recording was carried out while stimulating with a 90 mm round coil placed over different scalp electrode positions. Both the 21 standard recording electrode positions and the stimulus scalp positions were based on the international 10-20 system. The coil was placed with the recording electrode sitting in the centre of the coil, as illustrated in the figure below (Figure 2.1).

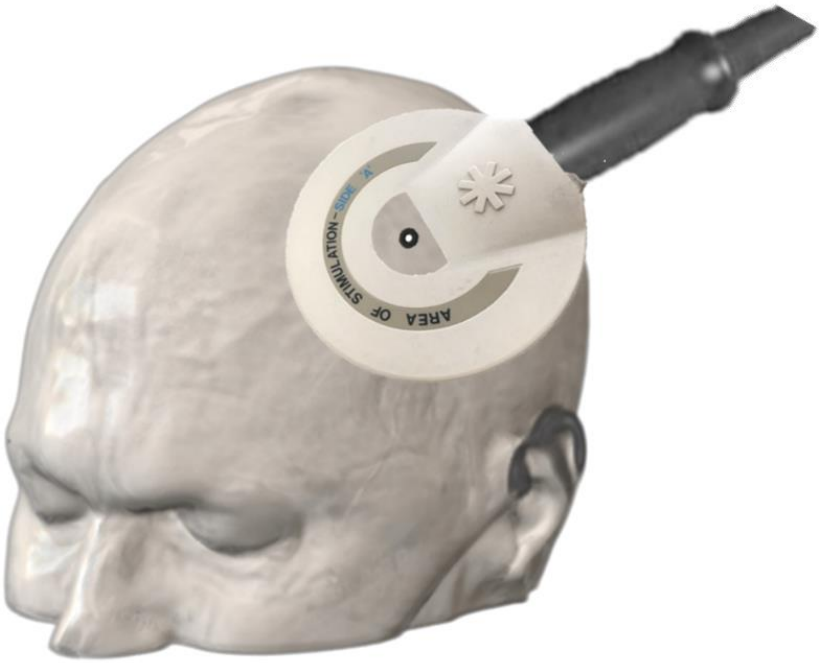


Figure 2.1. Placement of the coil. The recording electrode is located in the centre of the coil. The head model was provided by Dr Valentin (KCL).

The stimulating round coil was positioned with the centre of the coil placed over fifteen different scalp electrode positions of the standard international 10-20 system (F3, F4, T3, T4, T5, T6, P3, P4, O1, O2, C3, C4, Fz, Cz and Pz). The coil was carefully positioned to ensure that the direction of the intracranially induced current would be the same when stimulating equivalent scalp positions in the right and left hemispheres. The electrodes located in the right hemispheres were stimulated while placing the coil on the scalp with side A visible, which induces the current flow in the clockwise direction. Conversely, when the coil was flipped over to stimulate the left hemisphere,

side B was visible to ensure the same flow direction. The central positions (Fz, Cz, PZ) were stimulated both clockwise (A) and anticlockwise (B). As the three central channels were stimulated in two different directions (A/B), 18 stimulation channels are considered in the study, six central channels (FzA/FzB, CzA/CzB, PzA/PzB) plus the remaining 12 channels. At each scalp stimulation site, fifteen consecutive single TMS pulses separated by 5 seconds inter-stimulus intervals were delivered at 1.2 times the subject's resting cortico-motor threshold (Badawy et al., 2007, Valentin et al., 2008).

The level of contentiousness was controlled online by performing the TMS-EEG experiment with the patient relaxed with eyes closed and assessing the presence of alpha rhythm during the experiment. Acoustics mask was not implemented. A visual artefact rejection was done off- line. These EEG artefacts were identified visually in the off-line analysis, according to the standard EEG criteria: symmetrical positive deflections at the prefrontal electrodes (eye blinks), irregular high-frequency activity (muscle) and low-frequency high-amplitude deflections with no clear anatomical congruence (movement). No other artefact rejection techniques were implemented.

This protocol was based on previous experience with single-pulse electrical stimulation in epilepsy surgery candidate patients assessed with intracranial electrodes (Valentin et al., 2002, Valentin et al., 2005b, Valentin et al., 2005a) and in a previous pilot TMS study at KCL (Valentin et al., 2008).

C.2.1.5. TMS-EEG protocol optimisation

In order to determine the best TMS stimulation positions, multiple sites were tested, covering most of the brain surface in a cohort of ten volunteers and ten patients. Initially, all the EEG 10-20 international standard electrode positions, with the

exception of A1, A2, were used for TMS stimulation. Due to participant discomfort, positions F7, F8, Fp1, Fp2 were not implemented in the final TMS stimulation protocol.

C.2.1.6. Evaluation of the patient acceptability for the duration of the final TMS-EEG protocol.

A brief feedback questionnaire was completed by all the patients after the recording in order to establish the most suitable TMS-EEG protocol in terms of patient comfort and time optimisation without compromising the reliability of inducing TMS-EEG responses based on the experience of the studied subjects. The questions are listed below.

Q1: Would you do the TMS study again if it was proven to be clinically useful for the management of your condition?

Q2: Would you prefer to come for the TMS-EEG test as a separate appointment on a different day, or would you rather have both the routine sleep-EEG and TMS-EEG test on the same day?

Q3: On a visual analogue scale (VAS) from 1 to 10, 10 being the worst, the subjects were asked to rate the level of discomfort during the TMS study. For illustration purposes, the level of discomfort was classified as mild (VAS:1-3), tolerable (VAS: 3-5), distressing (VAS: 5-7) and unbearable (7-10).

Q4: Was the test procedure properly explained to you? If not, how can we improve it?

C.2.2 Qualitative analysis of the TMS evoked EGG responses

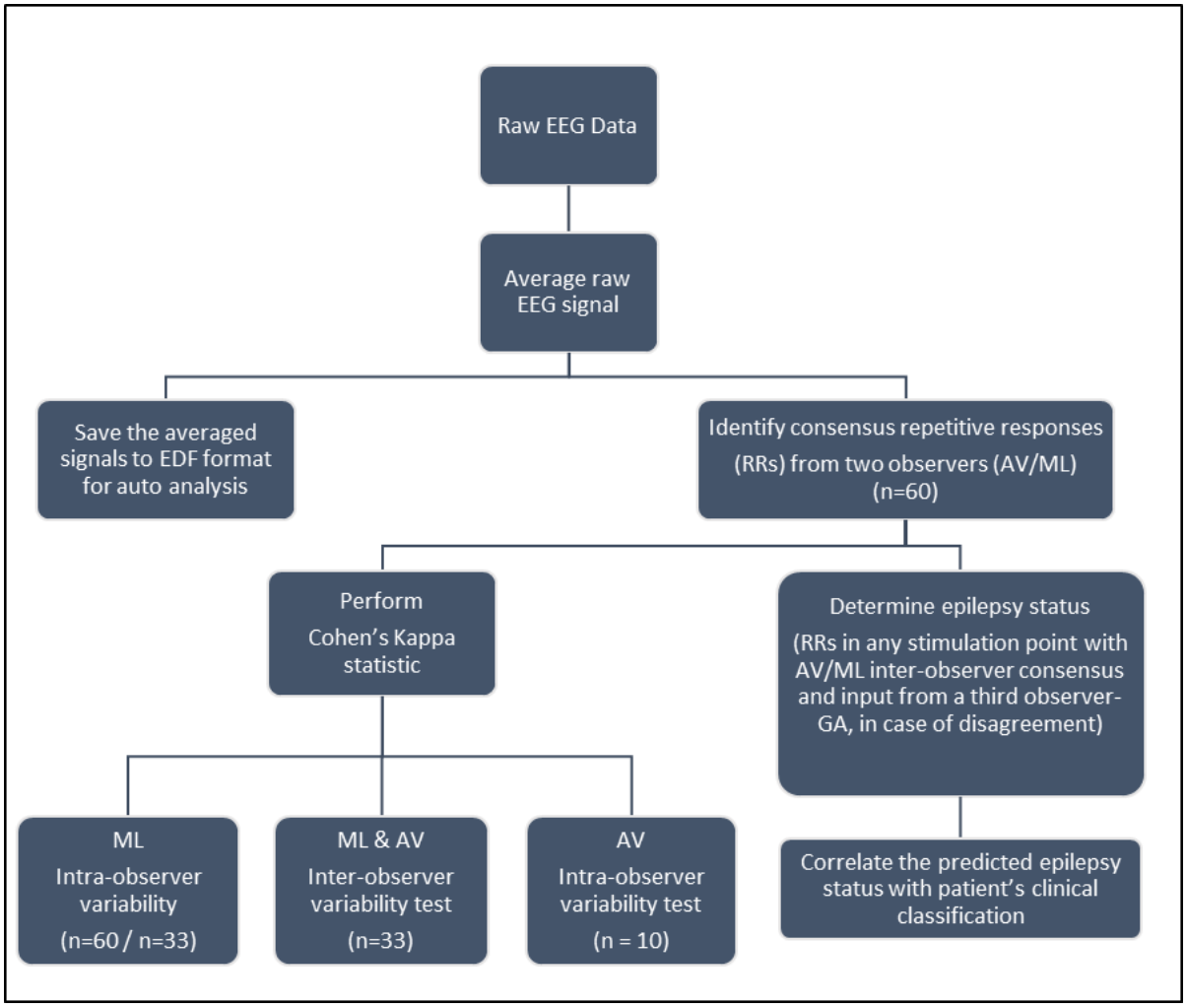


Figure 2.2. Qualitative analysis experiment flow-chart. AV, Antonio Valentin; GA, Gonzalo Alarcon; ML, Marian Lazaro. After collecting the TMS-EEG data, the visual analysis was performed in both the TMS-EEG and the baseline EEG recorded before the TMS study. The number of interictal discharges was quantified in both the baseline EEG and TMS-EEG to establish a numerical comparison in the amount of interictal activity between the two procedures. A subsequent second step in the visual analysis of TMS-EEG data was done after the averaging of the TMS evoked responses to study the repetitive responses (RRs). The RRs were stored in EDF format for subsequent quantitative analysis (see Chapter C.2.3). The visual identification of the RRs signals was followed by an intra-observer and inter-observer variability study to assess the reliability of the visual analysis. The TMS-EEG study was considered positive or indicative of epileptic trait if RRs were identified in at least one stimulation area, and following this criterion, the sample was subdivided on TMS-EEG grounds into two groups: subjects with a high likelihood of epilepsy given a positive TMS-EEG or

subjects with no epilepsy if the TMS-EEG results were negative. The TMS-EEG predicted epilepsy status was at a later stage correlated with the final clinical classification.

C.2.2.1 Preliminary visual analysis of the TMS-EEG and sleep-deprived EEG (baseline EEG) recordings.

The TMS-EEG and the sleep-deprived EEG (baseline EEG) recordings were visually inspected for epileptiform activity, interictal epileptiform discharges (IEDs), such as spikes, sharp waves, spike-poly spike and slow wave discharges. The number and position of the IEDs were annotated. Nonspecific interictal discharges (NSDs) of uncertain clinical significance were also annotated separately.

In order to avoid the confounding factor of the different time lengths between the baseline EEG and the TMS-EEG recordings, the number of discharges was divided by the total recording time length in minutes of both tests, resulting in two numerical values of the number of discharges per minute of recording. To compare the duration of the IEDs between baseline EEG and TMS-EEG, the total time duration of all runs in milliseconds was divided by the total recording time length in both tests, rendering the numerical values of discharge-time (in milliseconds) per minute of recording. The median time duration in milliseconds was also calculated for IEDs/NSD. The results were compared between baseline EEG and TMS-EEG.

In patients who showed IEDs and/or NSDs in the baseline EEG, a comparison of the visual finding was made between the TMS- EEG and the baseline EEG to assess the changes in the number, sum-duration and median duration of IEDs and NSDs in the TMS study by means of a matched paired comparison performed by parametric paired t-test.

In order to compare the effect of TMS on the discharges depending on clinical classification, an independent comparison between epileptic and non-epileptic subgroups was performed by looking at the arithmetical difference in the number, sum duration and median duration of the NSDs between baseline EEG and TMS-EEG. This arithmetical difference was calculated by subtraction of the aforementioned values (number, sum-duration, and median duration) on TMS-EEG minus the values in the baseline EEG in each subject. The comparison between patient groups (epilepsy, no-epilepsy) was performed on the arithmetical subtraction values (TMS-EEG minus baseline EEG) of the number, sum-duration, and median duration using the independent sample t-test. All statistical analysis was performed using the R statistic package.

C.2.2.2 Averaged EEG signal processing and analysis

The EEG signal was first re-referenced to common average reference and down-sampled to 1012 Hz. In order to increase the signal to noise ratio of the scalp EEG responses to TMS, signals in four seconds epochs spanning from 2 seconds pre-TMS stimulus to 2 seconds post-TMS stimulus were averaged using the average function of the TMS-EEG software (ASA-LAB system). The EEG signal-averaging procedure was carried out for each stimulation point by synchronising the EEG recordings with the TMS stimulus marker. The epochs contaminated with artefacts due to eye blinks, excessive muscle or movement artefacts were rejected. These EEG artefacts were identified visually according to the standard EEG criteria: symmetrical positive deflections at the prefrontal electrodes (eye blinks), irregular high-frequency activity (muscle) and low-frequency high-amplitude deflections with no clear anatomical congruence (movement). Epochs with IEDs or NSDs were also rejected. The

averaged EEG signals were saved in both European Data Format (EDF) and ASA Lab format for downstream quantitative analysis.

C.2.2.3 Analysis criteria for TMS evoked responses

The TMS evoked responses are classified into early responses (ERs) and late responses (LRs) that are subdivided into delayed responses (DRs) and repetitive responses (RRs) (Valentin et al., 2008).

The TMS evoked responses occurring within 200 msec post-TMS stimulation are defined as early responses (ERs). These ERs have also been recorded in healthy controls and will not be part of the study.

Delayed responses (DRs) methods analysis

The DRs are spike-like waveforms seen in the unprocessed EEG data prior to the average procedure, 100 milliseconds to 1 second following the TMS stimulation (Figure 2.3). The delayed responses were visually assessed through all the recording channels. In order to consider IEDs as DRs elicited by TMS, a non-parametric two-tailed sign test was used comparing IEDs noted during periods of 1 second before and after TMS stimulation ($p<0.05$) (Valentin et al., 2008).

Repetitive responses (RRs) methods analysis

The RRs are late responses seen after TMS stimulation at some stimulation positions following the averaging of the TMS-EEG recordings synchronised to the TMS stimulation artefact. The RRs definition criteria is a new-onset repetitive rhythmic activity, clearly different from the background EEG, showing a significant increase in amplitude (approximately 50%) in the low-frequency ranges (generally, theta or delta), seen between 200-250 milliseconds to 2 seconds, following the TMS stimulation and

appearing at least in three out of the 21 recording EEG recording channels at a given stimulation point. In order to consider these new rhythms as RRs, they should not appear with stimulation at the contralateral symmetrical position (Figure 2.4) (Valentin et al., 2008).

A minimum of at least ten epochs was considered necessary to ascertain the presence of repetitive responses (RRs); therefore, each averaged recording consisted of a minimum of 10 to a maximum of 15 epochs. The TMS-EEG averaged late responses, or repetitive responses (RRs), were visually inspected initially by two observers (ML, AV) through all the recording channels and stimulation points. The result was recorded as 0 and 1, in which 1 denotes the presence of the new rhythms. The RRs reported by both examiners were considered to be true positive. The RRs reported only by one observer (ML or AV) were assessed and classified by a third observer (GA).

TMS-EEG recordings with new-onset repetitive rhythmic activity in three or more recording channels at any given stimulation point were categorised as abnormal or positive TMS studies for RRs. This definition criteria of RRs was established by the aforementioned work of Valentin et. al. describing RRs in focal epilepsy. The association between the positive TMS study for RRs and the final clinical classification (epilepsy/ no-epilepsy) was evaluated by Fisher's exact test and Phi coefficient of correlation (Akoglu, 2018) for qualitative categorical data (psych R package) and visualised by correlation matrix heatmap (corrplot R package).

Combined late response (RRs and/or DRs) methods analysis

The presence of TMS-EEG combined late responses (RRs and/or DRs) was studied among epileptic and non-epileptic patients. Amplitude, distribution, morphology, latency, duration, frequency and incidence of these responses induced by TMS

stimulation at each site were also compared between groups, looking for responses associated with epilepsy. In patients where the first baseline EEG was normal, the diagnostic value of TMS-EEG was evaluated against the final diagnosis 12 months after the initial consultation in the First Seizure Clinic based on the clinical history, the assessment, the tests and the responsiveness to treatment. The association between the late responses and the final clinical classification was evaluated by Fisher's exact test.

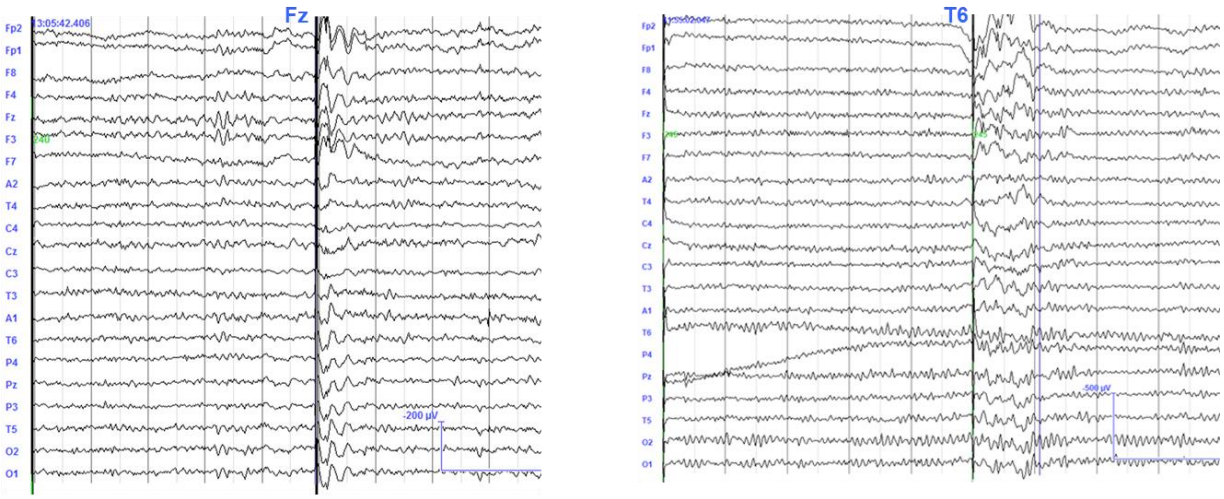


Figure 2.3. Delayed TMS-EEG responses. The delayed responses (DRS) were seen in TMS-EEG recordings without averaging following two single TMS stimulations (Fz and T6). Delayed responses were seen mainly over frontal areas (i.e., Fp2, Fp1, Fz, F3, F8 and F7 recording channels) after TMS stimulation of the midline frontal and temporal areas (Fz and T6). The vertical lines indicate the timing of the TMS pulse. The area where the TMS was applied is indicated above the EEG traces.

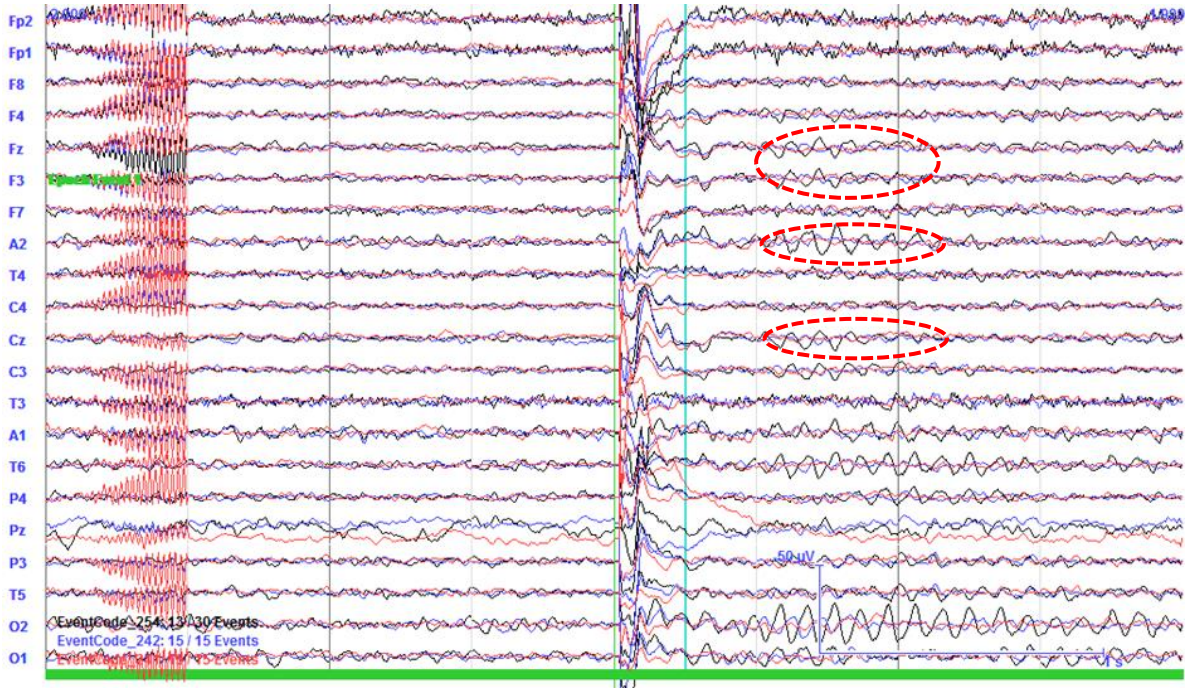


Figure 2.4. Repetitive TMS-EEG responses after averaging the TMS-EEG recordings. The average of the EEG synchronised to 15 TMS stimulation pulses at Cz or Pz does not show significant post-stimulus background changes in any of the 21 displayed EEG recording channels. **B.** On the same subject, the average of 15 TMS stimuli synchronised at Fz shows repetitive TMS-EEG responses in the recording channels (Fz, F3, A2, Cz and C3). Over the aforementioned channels, the RRs waveforms appear clearly distinctive from the background EEG following the TMS stimulation, as brief alpha frequency runs with a significant increase in the amplitude in comparison to the pre-stimulation background. The repetitive responses have been enclosed within circles. The vertical green lines indicate the timing of the TMS pulse. The area where the TMS was applied is indicated above the EEG traces. The channels in black, blue and red colour represent stimulation in Fz, Cz and Pz, respectively.

C.2.2.4. Intra-observer and inter-observer variability (Cohen's Kappa analysis)

The identification of repetitive responses in the study subjects was carefully performed by two independent observers (Marian Lazaro: ML and Antonio Valentin: AV). The results were dichotomised into 0 and 1, in which 1 represents the presence of a new-onset repetitive rhythm and 0 represents the lack of the repetitive rhythm in the recording channels after TMS stimulation. Each observer analysed the TMS-EEG responses twice to assess the overall intra-observer and inter-observer variability and the discrepancy at the different stimulation points, recording channels and at the patient level.

For inter-observer variability (ML/AV), the RRs results from thirty-three patients with a complete TMS-EEG dataset were analysed. A binary matrix was constructed for the analysis of the Cohen's Kappa Coefficient (McHugh, 2012). Each column denotes a recording channel in a stimulation point (the intersections between the 18 stimulation areas and the 21 recording channels are defined as the 378 TMS-EEG data points or columns), and each row represents a patient (column=378, row=33, Figure 2.5A, step 2 and 2.5B). A vector consisting of 12474 data points (21 x 18 x 33, Supplementary Figure 2.5A, step 3 and 2.5B) derived from each observer was used to calculate the overall Cohen's Kappa coefficient (Figure 2.5C). The same methodology was used to calculate the intra-observer variability (ML/ML and AV/AV).

For disagreement in each data point derived from the combination of the 18 stimulating points (SP) per 21 recording channels (RC), each column from the observers was compared. This is to give a general view of which channel and stimulation point may yield more discrepancy, indirectly indicating the complexity of the

electroencephalographic responses in a particular channel/stimulation point (Figure 2.6).

The total discrepancies in each subject were scrutinised to rule out if the difference was attributed to a particular subject. All statistical analyses and graphics were performed using psy (ver 1.1), corrplot (ver 0.77) and ComplexHeatmap (ver 1.120) R packages.

C.2.2.5. Correlation of TMS-EEG and clinical classification

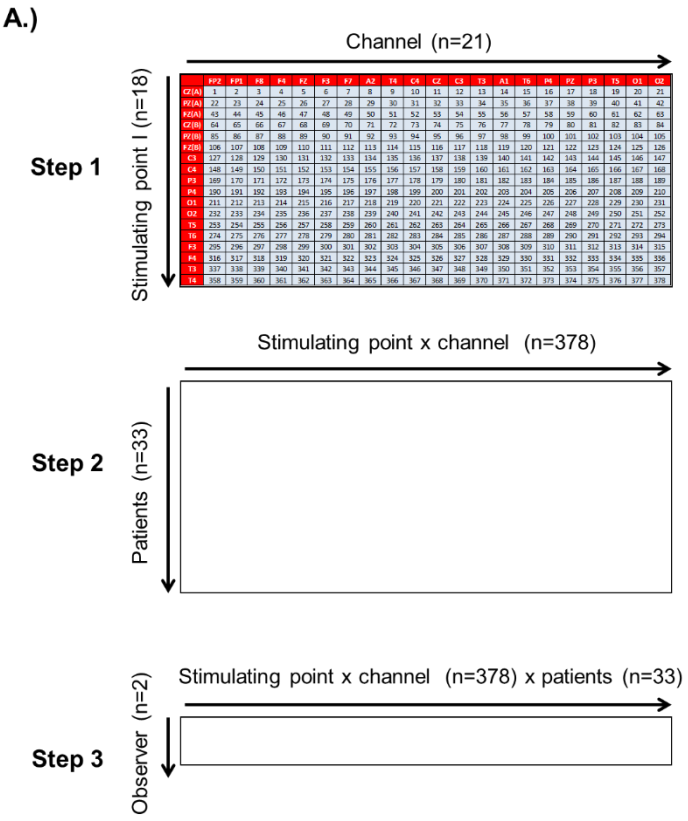
The patients' clinical classification was performed by Prof Koutroumanidis at 12 months, based on the clinical history, the occurrence of subsequent seizures, the presence of abnormalities in the baseline EEGs or in the other tests (neuroimaging, tilt table/cardio investigations) and the responsiveness to treatment. At that time, Prof Koutroumanidis was blinded to the results of the TMS-EEG studies. As TMS-EEG is presently a research technique, the results from TMS-EEG were not considered for the clinical diagnosis of epilepsy in the recruited patients. Patients were classified as epileptic with a subclassification of focal or generalised epilepsy and non-epileptic with a subclassification of vasovagal syncope (VVS) and psychogenic non-epileptic seizures (PNES).

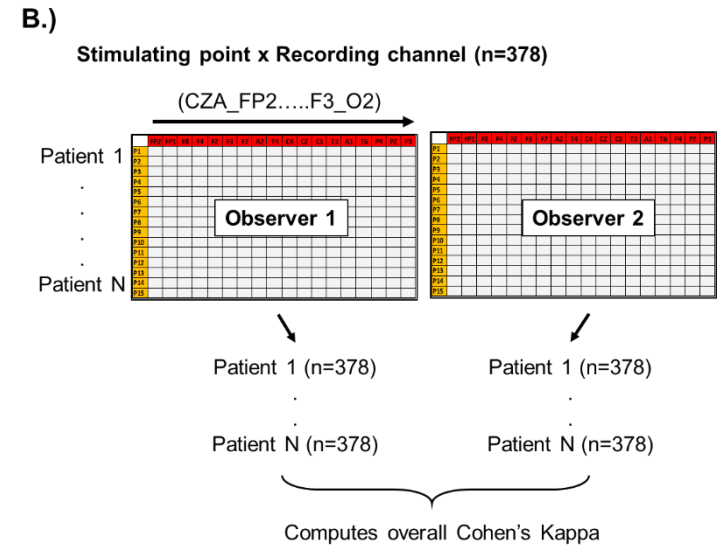
Different qualitative TMS-EEG features were considered for the correlation of TMS-EEG and the clinical condition:

1.) Presence or absence of TMS-EEG late responses subclassified into delayed responses (DRs) and repetitive responses (RRs) in patients classified as highly probable to be epileptic in the follow up 12 months clinic.
2.) Presence of absence of TMS-EEG late responses, comparing normal or abnormal baseline EEG in epileptic patients.

3.) Presence or absence of specific characteristics in the TMS-EEG late responses shown in different epileptic sub-groups regarding amplitude, distribution, morphology, latency, duration, frequency and incidence of responses.

4.) Presence or absence of TMG-EEG late responses in participants considered non-epileptic subjects in the follow-up clinic.





C.)

Confusion table

		ob1	
		0	**1**
ob2	**0**	12056	153
	1	189	76

Kappa coefficient : 0.294

Figure 2.5. Methodology for computation of the overall Cohen's Kappa coefficient to obtain the overall Intra-observer's and Inter-observer's variability (Cohen's Kappa analysis). **A.** The TMS-EEG data was transformed to create binary matrices suitable for the study. **Step 1,** shows an example of the RRs data displayed for a subject in a matrix in which each column represents one of the 21 recording channels (RC) and each row represents one of the 18 TMS stimulating points (SP). **Step 2**, the data from step 1 was linearised to create a new matrix in which each column represents one of the 378 data points generated from the combination of the 18 stimulating points (SP) x 21 recording channels (RC), and each row represents a variable number of subjects depending on the particular study performed (60 ML/ML; 33 ML/AV). **Step 3**, the data from step 2 is further transformed two create two vectors, as shown below in Figure 2.5.B. **B.** Two examples of binary matrices with the data from observers 1 and 2 as described in step 2. After the matrices were linearised and a vector consisting of 22680 data points (21 RC x 18 SP x 60 P) or 12474 data points (21 RC x 18 SP x 33 P) derived from each observation (obs1/obs2) was used to calculate the overall Cohen's Kappa coefficient. **C.** The overall Kappa coefficient computed results are represented in a confusion table, 0=no repetitive new rhythm, 1= repetitive new rhythm.

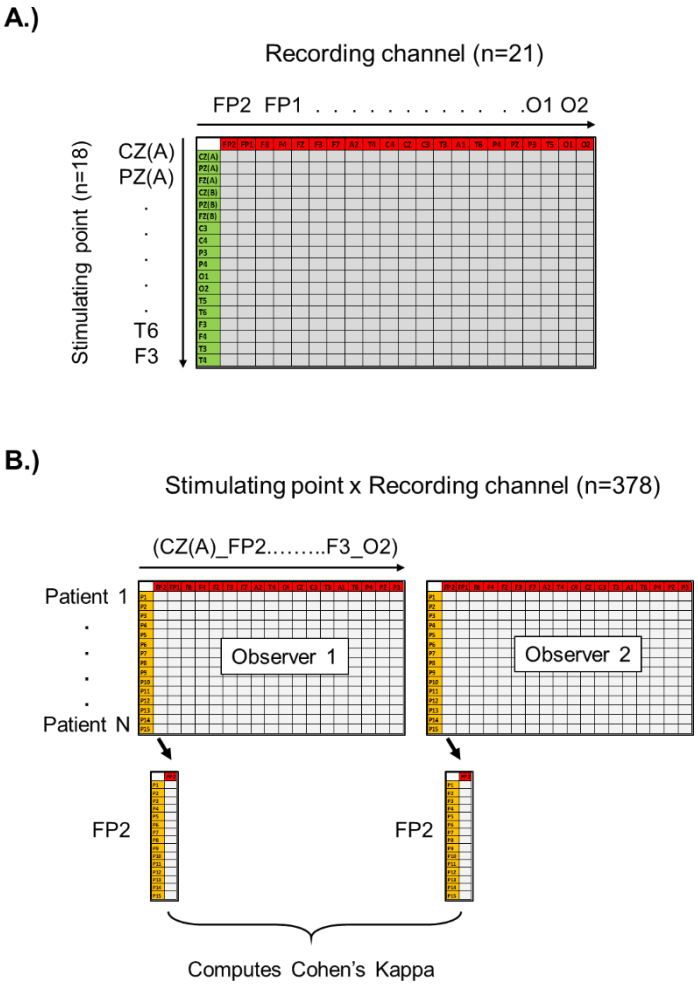


Figure 2.6. Methodology for computation of the Cohen's Kappa coefficient variability to obtain the Intra-observer's and Inter-observer's variability at each specific data point (Cohen's Kappa analysis). **A.** Matrix displaying the data for a subject, each cell representing one of the 378 data points derived from the combination of 18 stimulating points (SP) x 21 recording channels (RC). **B.** The data from matrix A was linearised as described in Figure 2.5. step 2, resulting in matrix B, in which the columns represent the 378 data points and the rows represent the subjects. This time the Cohen's Kappa analysis was performed by comparing each corresponding column between observer 1 and observer 2 to calculate the variability/ disagreement at each of the 378 individual data points. This analysis generates 378 specific Kappa coefficient values corresponding to the 378 individual data points. The data obtained is graphically represented in a Cohen's Kappa heatmap in which each square will represent the Kappa coefficient for each data point, see chapter C.3.6.7. Figure 3.16.

C.2.3. Quantitative analysis of the TMS-EEG evoked responses

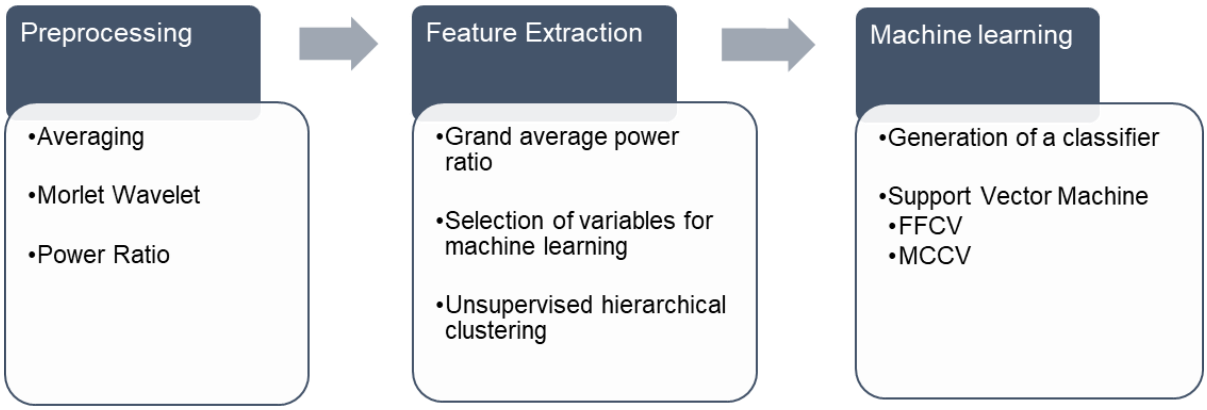


Figure 2.7. Quantitative analysis experiment flowchart. The flowchart shows the workflow of the quantitative analysis of the TMS-EEG data. The first stage consisted of the pre-processing of the TMS-EEG raw data by the averaging process explained in the material and methods qualitative analysis chapter. Subsequently, the continuous EEG signals were transformed from the time domain to the time-frequency domain by the Morlet Wavelet transformation technique that calculates the mean power in the different frequency bands. Afterwards, the power ratio before and after stimulation was calculated. The second stage was the extraction of the relevant features to characterise the epileptic sample. In order to do this, the power ratio grand average was calculated to compare epilepsy and non-epilepsy groups. Also, the feature selection of the important variables for machine learning (epilepsy-associated frequencies at a given stimulation and recording channel) was performed by the Wilcoxon rank-sum test. To investigate if these selected variables were associated with the clinical classification, an unsupervised hierarchical clustering analysis was performed. The last step was the generation of the epilepsy prediction classifier using two cross-validation strategies, Monte Carlo Cross-Validation (MCCV) and Five-Fold Cross-Validation (FFCV), to support the machine learning algorithm.

C.2.3.1. Study cohort

Of the sixty subjects (twenty-seven epileptic and thirty-three non-epileptic subjects based on clinical criteria) who comprised the cohort for the qualitative study, only forty-three had a complete TMS study, while seventeen of the participants (eight epileptic and nine non-epileptic) had an incompletely recorded study. In these cases, the TMS protocol was not fully undertaken due to the subjects' intolerance to the test or technical recording pitfalls, which precluded TMS stimulation in one or more of the 18 stimulation channels. The cases in which the TMS study was incomplete were rejected for the quantitative analyses. Upon visual inspection of the 43 completed TMS studies, eight studies were considered excessively noisy for the purpose of the quantitative analysis. This was due to the excessive number of recording channels with artefacts, and these were also rejected for the quantitative study. Therefore, 35 subjects (16 epileptic and 19 non-epileptic) with complete and artefact free TMS recordings were selected for the quantitative analysis (Table 2.1).

In order to test if the TMS optimises disease detection by the classifier in comparison to the EEG alone, a sham study was performed. The sham study was performed in the same 35 subjects-cohort utilising the baseline EEG recorded before the TMS session. In the Sham, the markers for the stimulation channels and stimulation modalities were randomly assigned, with no actual TMS stimulation taking place in the designated sham stimulation markers.

C.2.3.2. Wavelet transformation

A bespoke MATLAB script was written to transform the continuous signals into the time-frequency domain and power ratio before and after the TMS stimulation. In brief, this script loads the EDF file and sets up the epoch of interest at 1.5 seconds, both

pre-stimulation and post-stimulation. The sample of interest is 1.5 seconds of EEG-TMS data, both pre- and post-stimulation. The offset for pre-stimulation is from the beginning of the file, and the offset for post-stimulation is 200 milliseconds after the stimulus (or the event marker for the Sham). The analysis of the first 200 milliseconds post-TMS stimulation was omitted for two main reasons. First, to ensure the quantification of actual TMS-related EEG changes and to avoid contamination from auditory and somatosensory evoked potentials that, to a certain degree, may contribute to the early EEG signal changes seen after TMS stimulation. Furthermore, the quantitative analysis aims to extend the previous visual examination of the TMS-evoked late responses, specifically the repetitive responses that, as defined in the literature, would appear 200 milliseconds after the TMS stimulation.

The pre-stimulation and post-stimulation segments were transferred to the wavelet domain by means of continuous wavelet transform (Morlet mother wavelet). The power ratio for each individual recording channel was then calculated as power ratio= power post-stimulation/ power pre-stimulation for every frequency across the 1 ~ 42 Hz frequency range. Frequency bins are 0.5 Hz (Figure 2.8). To calculate the power ratio in the traditional banding (Delta, Theta, Alpha, Beta and Gamma frequencies), the arithmetic mean across the 0.5 frequency bins (V1-V83) was calculated as follows: Delta (bins: V1-V7, frequencies: 0.5-3.5); Theta (bins: V8-V15, frequencies: 4-7.5) Alpha (bins: V16-V27, frequencies: 8-13.5); Beta (bins: V28-V60, frequencies: 14-30) and Gamma (bins: V61-V83, frequencies: 30.5-42). First, the power ratio calculation is done for each recording channel separately, and the average of all the recording channels is done as a final step to calculate the average power ratio in each stimulation area.

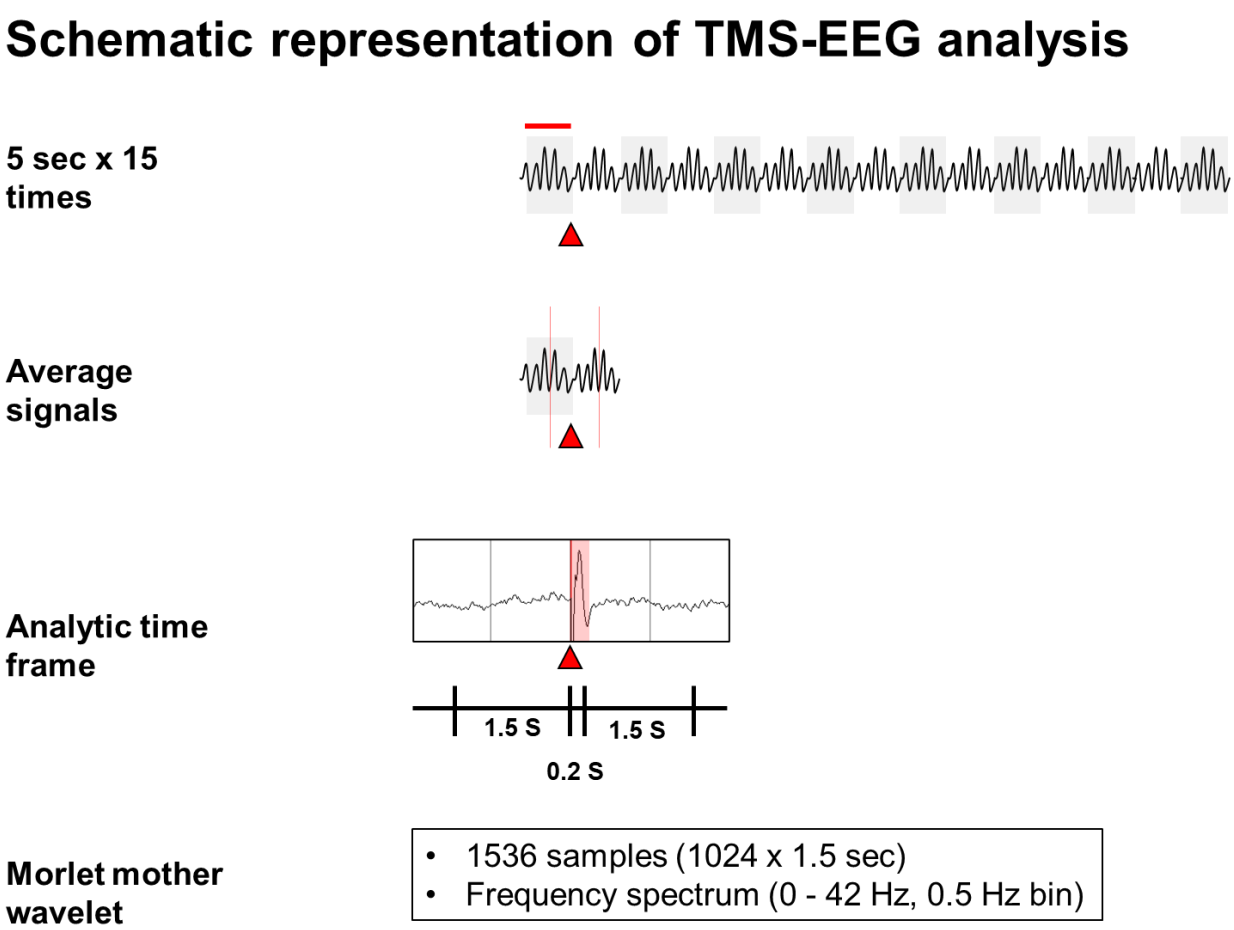


Figure 2.8. Schematic representation of the pre-processing of the TMS-EEG signals and transformation of the signal with Morlet mother wavelet script.

C.2.3.3. Grand average power-ratio

The power ratio of twenty-one recording channels was averaged in each of the 18 stimulation points across the 1 ~ 42 Hz frequencies. The grand average power ratio is the mean average power ratio in each stimulation point in a cohort. The mean and the 95% confidence interval of 35 participants (16 epileptic and 19 non-epileptic) were computed and visualised using Rmisc (1.5) and ggplot2 (3.2.1) R package.

C.2.3.4. Selection of epilepsy-associated features for machine learning models

Metadata structure

In order to ascertain if any recording channels and stimulation points were associated with epilepsy at a particular frequency band and if these features could be used to predict epilepsy, the power ratio of each recording channel at each stimulation area (termed recording point) was analysed. A TMS activated recording channel (TARC), also referred to as recording point (RP) as an abbreviation, is defined as a specific recording channel during TMS stimulation at a specific stimulation area. The combination of the 21 recording channels during stimulation at each of the 18 stimulation sites results in 378 recording points. A metadata table with clinical status was constructed for the analysis, consisting of 1890 variables/columns (5 frequency bins x 21 channels x 18 stimulation points) and 35 subjects/rows. The 5 frequency bins containing the delta, theta, alpha, beta and gamma frequency bands. Therefore, each variable represented a particular data recording point whose value was the power ratio at a particular frequency band (V, total 5 bins) recorded in a particular channel (CH, total 21) upon TMS stimulation of a specific stimulation point (SP, total 18). This dataset comprised epileptic (16) and non-epileptic (19) subjects (Figure 2.9).

Exploration of epilepsy-associated variables for the generation of a classification model

The non-parametric Wilcoxon Rank Sum test was used to explore the possibility of finding variables associated with the disease. A shortlist of 60 variables that had a differential power ratio scale between epilepsy and non-epilepsy with $p \leq 0.05$ were selected from the 1890 data points. Multiple comparison correction was performed over these 60 variables with Bonferroni and Benjamini-Hochberg (False Discovery Rate) methods and an adjusted p-value smaller than 0.05 was considered statistically significant.

Clustering analysis

The preliminary variable selection with the Wilkinson rank-sum test method failed Bonferroni correction. Therefore the 60 variables were subjected to z-score (using the scale command in the base R package, version 3.6.0) normalisation prior to unsupervised hierarchical clustering analysis using Euclidean distance matrix and complete linkage. The association between epilepsy and non-epilepsy and the power ratio scale patterns were visualised by ComplexHeatmap R package (2.2.0 version).

When these variables were explored with unsupervised clustering analysis, the result showed that these variables were indeed associated with the disease. Therefore, a new methodological approach to variable selection was introduced, using the FFCV and MMCV methods of the CMA R package. In this new method, only the variables with a p-value ≤ 0.05 in at least two bootstrapping datasets in both FFCV and MCCV were selected. This method is more stringent, reducing the number of variables and removing the variables that are weakly associated with epilepsy (see section C.2.3.5).

C.2.3.5. Machine learning-based classification

Selection of epilepsy-associated features

The selection of epilepsy-associated features was carried out by in-build commands GenerateLearningsets and GeneSelection in the CMA package (Synthesis of microarray-based classification. R package version 1.50.0). The Wilcoxon Rank Sum test was used on the 1890 variables as a means to filter the most relevant to the less relevant variables through two re-sampling methods, Five-fold cross-validation (FFCV) and Monte Carlo cross-validation (MCCV), to generate 100 simulation datasets (Patro, 2021). The two resampling methods employ iteration and stratification to generate 100 simulation datasets (Figure 2.10). To apply a stringent selection protocol, the variables were then ranked according to their p-value. Variables with a p-value ≤ 0.05 appearing in at least ten simulation datasets in both FFCV and MCCV were compared, and only the overlapped variables were deemed suitable to generate the classifier for the epilepsy prediction model. With this method, the variables weakly associated with epilepsy were removed from the original 1890 variables, and the classifier was built upon this reduced number of selected variables (Krzywinski and Altman, 2014, McLeod, 2019).

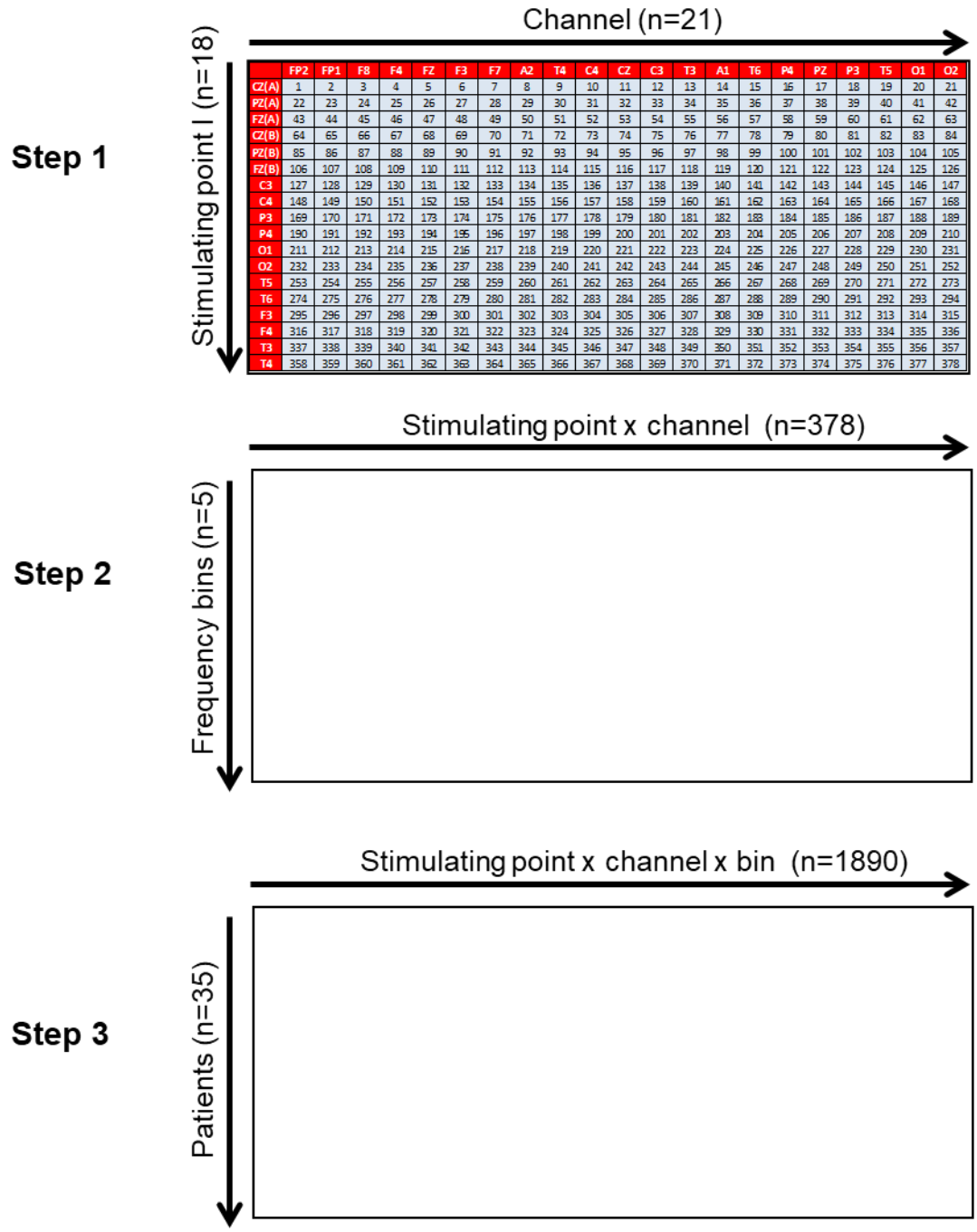
Generation of epilepsy prediction classifier

The machine learning R package (CMA) and the aforementioned variables derived from epilepsy-associated features were used to generate the classifier as an epilepsy prediction model, using support vector machine (SVM) with linear kernel algorithm and FFCV and MCCV re-sampling methods (Chang, 2002, Scholkopf et al., 2000, Slawski et al., 2008).

Once the classifier was constructed, an assessment of the classifier performance was made with both FFCV and MCCV re-sampling methods. This was evaluated by the average predicted probability for the correct classification, sensitivity, specificity and misclassification rate in the test or validation dataset (total sample subtracted from the training set). The evaluation was run in 100 datasets, and the results were aggregated to become one final value to assess how well the classifier performed (Tharwat, 2018).

Classifier prediction of epilepsy in an independent cohort

An independent cohort of 37 participants recruited at KCH for a prior pilot study on focal epilepsy was used as a prediction dataset for the epilepsy classification models (14 non epileptic subjects, 23 patients with long-standing focal epilepsy). These participants were subjected to the same TMS-EEG protocol as the current study, and the study was undertaken by AV. The only differences were the use of a figure of eight coil instead of a round coil and the participants not being sleep deprived at the time of the TMS-EEG study. The TMS-EEG data was processed and arranged following the process previously described in the materials and methods sections C.2.2.2, C.2.3.2, C.2.3.4 (metadata structure) and C.2.3.5 (Generation of epilepsy prediction classifier).



	FP2	FP1	F8	F4	FZ	F3	F7	A2	T4	C4	CZ	C3	T3	A1	T6	P4	PZ	P3	T5	O1	O2
CZ(A)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
PZ(A)	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42
FZ(A)	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63
CZ(B)	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84
PZ(B)	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100	101	102	103	104	105
FZ(B)	106	107	108	109	110	111	112	113	114	115	116	117	118	119	120	121	122	123	124	125	126
C3	127	128	129	130	131	132	133	134	135	136	137	138	139	140	141	142	143	144	145	146	147
C4	148	149	150	151	152	153	154	155	156	157	158	159	160	161	162	163	164	165	166	167	168
P3	169	170	171	172	173	174	175	176	177	178	179	180	181	182	183	184	185	186	187	188	189
P4	190	191	192	193	194	195	196	197	198	199	200	201	202	203	204	205	206	207	208	209	210
O1	211	212	213	214	215	216	217	218	219	220	221	222	223	224	225	226	227	228	229	230	231
O2	232	233	234	235	236	237	238	239	240	241	242	243	244	245	246	247	248	249	250	251	252
T5	253	254	255	256	257	258	259	260	261	262	263	264	265	266	267	268	269	270	271	272	273
T6	274	275	276	277	278	279	280	281	282	283	284	285	286	287	288	289	290	291	292	293	294
F3	295	296	297	298	299	300	301	302	303	304	305	306	307	308	309	310	311	312	313	314	315
F4	316	317	318	319	320	321	322	323	324	325	326	327	328	329	330	331	332	333	334	335	336
T3	337	338	339	340	341	342	343	344	345	346	347	348	349	350	351	352	353	354	355	356	357
T4	358	359	360	361	362	363	364	365	366	367	368	369	370	371	372	373	374	375	376	377	378

Figure 2.9. Analysis methodology for metadata table generation and preliminary exploration of epilepsy-associated features. Wilkinson rank-sum test analysis was performed over the 1890 variables by comparing the mean value of these variables between epileptic and non- epileptic cohorts.

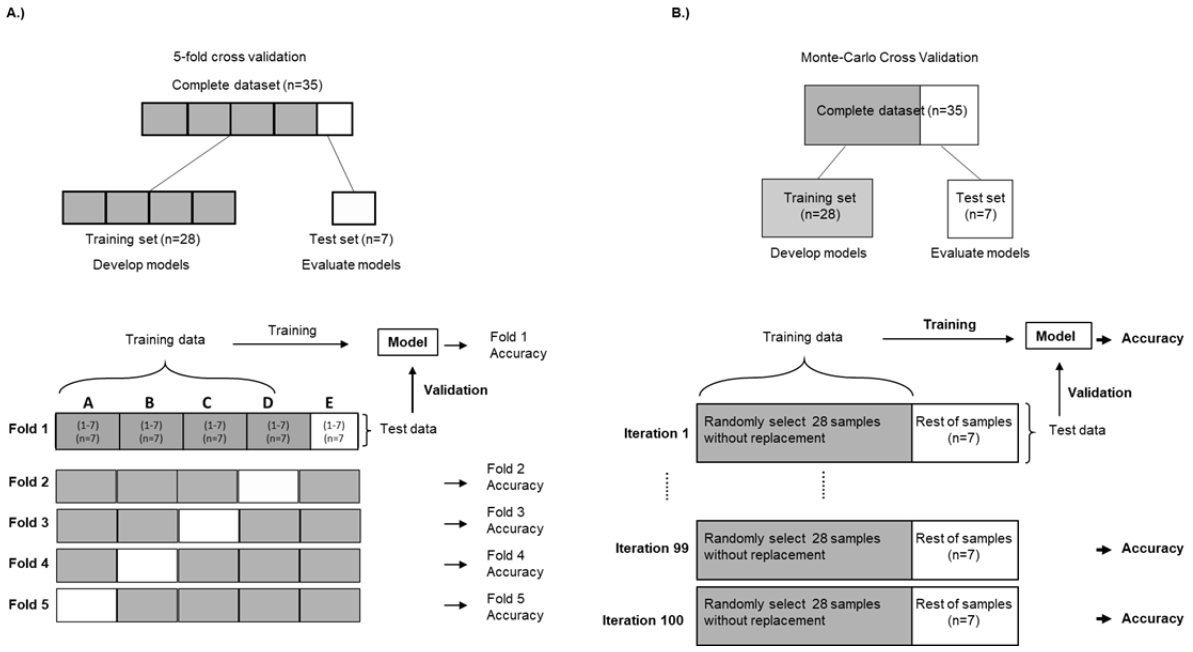


Figure 2.10. Illustration of the two resampling methods used, Five-fold cross-validation (FFCV) and Monte Carlo cross-validation (MCCV). A) Five-Fold cross-validation resampling method randomly divides the samples into five equal groups using 1/5 of the sample (n=7) for validation (test set) and 4/5 of the sample (n=28) for iteration (training set). The iteration was repeated 20 times in each fold in order to generate 100 datasets. **B)** In MCCV, the randomly selected 4/5 of the samples (n=28) constitute the training set, and the remaining 1/5 (n=7) is the test set. The iteration was repeated 100 times in each fold in order to generate 100 datasets.

Patient id	N=35	Artefacts	C.C.	FZA	PZA	CZA	FZB	PZB	CZB	O2	O1	T6	P3	P4	T5	T4	T3	C4	C3	F4	F3
20131118_1138	Y		NE	FZA	PZA	CZA	FZB	PZB	CZB	O2	O1	T6	P3	P4	T5	T4	T3	C4	C3	F4	F3
20140212_1504	Y		NE	FZA	PZA	CZA	FZB	PZB	CZB	O2	O1	T6	P3	P4	T5	T4	T3	C4	C3	F4	F3
20140618_1111	Y		NE	FZA	PZA	CZA	FZB	PZB	CZB	O2	O1	T6	P3	P4	T5	T4	T3	C4	C3	F4	F3
20140618_1540	Y		NE	FZA	PZA	CZA	FZB	PZB	CZB	O2	O1	T6	P3	P4	T5	T4	T3	C4	C3	F4	F3
20140630_1111	Y		NE	FZA	PZA	CZA	FZB	PZB	CZB	O2	O1	T6	P3	P4	T5	T4	T3	C4	C3	F4	F3
20140728_1514	Y		NE	FZA	PZA	CZA	FZB	PZB	CZB	O2	O1	T6	P3	P4	T5	T4	T3	C4	C3	F4	F3
20141103_1041	Y		NE	FZA	PZA	CZA	FZB	PZB	CZB	O2	O1	T6	P3	P4	T5	T4	T3	C4	C3	F4	F3
20141105_1057	Y		NE	FZA	PZA	CZA	FZB	PZB	CZB	O2	O1	T6	P3	P4	T5	T4	T3	C4	C3	F4	F3
20141110_1452	Y		NE	FZA	PZA	CZA	FZB	PZB	CZB	O2	O1	T6	P3	P4	T5	T4	T3	C4	C3	F4	F3
20141124_1059	Y		NE	FZA	PZA	CZA	FZB	PZB	CZB	O2	O1	T6	P3	P4	T5	T4	T3	C4	C3	F4	F3
20150119_1040	Y		NE	FZA	PZA	CZA	FZB	PZB	CZB	O2	O1	T6	P3	P4	T5	T4	T3	C4	C3	F4	F3
20150608_1342	Y		NE	FZA	PZA	CZA	FZB	PZB	CZB	O2	O1	T6	P3	P4	T5	T4	T3	C4	C3	F4	F3
20151021_1121	Y		NE	FZA	PZA	CZA	FZB	PZB	CZB	O2	O1	T6	P3	P4	T5	T4	T3	C4	C3	F4	F3
20160118_1033	Y		NE	FZA	PZA	CZA	FZB	PZB	CZB	O2	O1	T6	P3	P4	T5	T4	T3	C4	C3	F4	F3
20140430_1108	Y		NE	FZA	PZA	CZA	FZB	PZB	CZB	O2	O1	T6	P3	P4	T5	T4	T3	C4	C3	F4	F3
20140514_1035	Y		E	FZA	PZA	CZA	FZB	PZB	CZB	O2	O1	T6	P3	P4	T5	T4	T3	C4	C3	F4	F3
20140818_1010	Y		NE	FZA	PZA	CZA	FZB	PZB	CZB	O2	O1	T6	P3	P4	T5	T4	T3	C4	C3	F4	F3
20140910_1038	Y		NE	FZA	PZA	CZA	FZB	PZB	CZB	O2	O1	T6	P3	P4	T5	T4	T3	C4	C3	F4	F3
20150309_1514	Y		NE	FZA	PZA	CZA	FZB	PZB	CZB	O2	O1	T6	P3	P4	T5	T4	T3	C4	C3	F4	F3
20140414_1238	Y		E	FZA	PZA	CZA	FZB	PZB	CZB	O2	O1	T6	P3	P4	T5	T4	T3	C4	C3	F4	F3
20140428_1105	Y		E	FZA	PZA	CZA	FZB	PZB	CZB	O2	O1	T6	P3	P4	T5	T4	T3	C4B	C3A	F4	F3
20140519_1010	Y		E	FZA	PZA	CZA	FZB	PZB	CZB	O2	O1	T6	P3	P4	T5	T4	T3	C4	C3	F4	F3
20140602_1039	Y		E	FZA	PZA	CZA	FZB	PZB	CZB	O2	O1	T6	P3	P4	T5	T4	T3	C4	C3	F4	F3
20140602_1504	Y		E	FZA	PZA	CZA	FZB	PZB	CZB	O2	O1	T6	P3	P4	T5	T4	T3	C4	C3	F4	F3
20140623_1028	Y		E	FZA	PZA	CZA	FZB	PZB	CZB	O2	O1	T6	P3	P4	T5	T4	T3	C4	C3	F4	F3
20140707_1419	Y		E	FZA	PZA	CZA	FZB	PZB	CZB	O2	O1	T6	P3	P4	T5	T4	T3	C4	C3	F4	F3
20140730_1051	Y		E	FZA	PZA	CZA	FZB	PZB	CZB	O2	O1	T6	P3	P4	T5	T4	T3	C4	C3	F4	F3
20140804_1105	Y		E	FZA	PZA	CZA	FZB	PZB	CZB	O2	O1	T6	P3	P4	T5	T4	T3	C4	C3	F4	F3
20140811_1218	Y		E	FZA	PZA	CZA	FZB	PZB	CZB	O2	O1	T6	P3	P4	T5	T4	T3	C4	C3	F4	F3
20150126_1503	Y		E	FZA	PZA	CZA	FZB	PZB	CZB	O2	O1	T6	P3	P4	T5	T4	T3	C4	C3	F4	F3
20150422_1120	Y		E	FZA	PZA	CZA	FZB	PZB	CZB	O2	O1	T6	P3	P4	T5	T4	T3	C4	C3	F4	F3
20150615_1032	Y		E	FZA	PZA	CZA	FZB	PZB	CZB	O2	O1	T6	P3	P4	T5	T4	T3	C4	C3	F4	F3
20160718_1001	Y		NE	FZA	PZA	CZA	FZB	PZB	CZB	O2	O1	T6	P3	P4	T5	T4	T3	C4	C3	F4	F3
20140630_1438	Y		E	FZA	PZA	CZA	FZB	PZB	CZB	O2	O1	T6	P3	P4	T5	T4	T3	C4	C3	F4	F3
20150121_1024	Y		E	FZA	PZA	CZA	FZB	PZB	CZB	O2	O1	T6	P3	P4	T5	T4	T3	C4	C3	F4	F3
20140120_1112			NE	FZA	PZA	CZA	FZB	PZB	CZB	O2	O1	T6	P3	P4	T5	T4	T3	C4	C3	N/A	N/A
20140507_1107			NE	FZA	PZA	CZA	FZB	PZB	CZB	O2	O1	N/A	P3	P4	N/A	T4	T3	C4	C3	F4	F3
20140512_1128			NE	FZA	PZA	CZA	FZB	PZB	CZB	N/A	N/A	N/A	P3	P4	N/A	N/A	N/A	C4	C3	N/A	N/A
20140804_1601			NE	FZA	PZA	CZA	FZB	PZB	CZB	N/A	N/A	N/A	P3	P4	N/A	N/A	N/A	C4	C3	N/A	N/A
20140908_1459			NE	N/A	PZA	CZA	N/A	PZB	CZB	N/A	N/A	T6	P3	P4	T5	N/A	N/A	C4	C3	N/A	N/A
20141029_1040			NE	FZA	PZA	CZA	FZB	PZB	CZB	N/A	N/A	N/A	P3	P4	N/A	T4	T3	C4	C3	N/A	F3
20150518_1059			NE	FZA	PZA	CZA	FZB	PZB	CZB	N/A	N/A	T6	P3	P4	T5	N/A	N/A	C4	C3	N/A	N/A
20151019_1047			NE	FZA	PZA	CZA	FZB	PZB	CZB	N/A	N/A	N/A	P3	P4	N/A	N/A	N/A	C4	C3	N/A	F3
20141015_1012			NE	FZA	PZA	CZA	FZB	PZB	CZB	O2	O1	T6	P3	P4	T5	T4	T3	C4	C3	N/A	N/A
20140331_1113			E	FZA	PZA	CZA	FZB	PZB	CZB	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	C4	C3	N/A	N/A
20140407_1114			E	FZA	PZA	CZA	FZB	PZB	CZB	O2	O1	N/A	P3	P4	N/A	T4	T3	C4	C3	F4	F3
20140714_1444			E	FZA	PZA	CZA	FZB	PZB	CZB	O2	O1	T6	P3	P4	T5	N/A	N/A	C4	C3	F4	F3
20141124_1518			E	N/A	PZA	CZA	N/A	PZB	CZB	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
20141201_1046			E	FZA	PZA	CZA	FZB	PZB	CZB	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	F4	F3
20141201_1434			E	FZA	PZA	CZA	FZB	PZB	CZB	O2	O1	T6	P3	P4	T5	N/A	N/A	C4	C3	F4	F3
20151214_1500			E	FZA	PZA	CZA	FZB	PZB	CZB	N/A	N/A	N/A	P3	P4	T5	N/A	N/A	C4	C3	F4	F3
20160125_1355			E	FZA	PZA	CZA	FZB	PZB	CZB	O2	O1	T6	P3	P4	T5	T4	T3	C4	C3	F4	N/A
20150710_1355			NE	FZA	PZA	CZA	FZB	PZB	CZB	O2	O1	T6	P3	P4	T5	T4	T3	C4	C3	F4	F3
20131125_1051			NE	FZA	PZA	CZA	FZB	PZB	CZB	O2	O1	T6	P3	P4	T5	T4	T3	C4	C3	F4	F3
20140512_1450			NE	FZA	PZA	CZA	FZB	PZB	CZB	O2	O1	T6	P3	P4	T5	T4	T3	C4	C3	F4	F3
20140829_1555			NE	FZA	PZA	CZA	FZB	PZB	CZB	O2	O1	T6	P3	P4	T5	T4	T3	C4	C3	F4	F3
20140609_1139			E	FZA	PZA	CZA	FZB	PZB	CZB	O2	O1	T6	P3	P4	T5	T4	T3	C4	C3	F4	F3
20140915_1104			E	FZA	PZA	CZA	FZB	PZB	CZB	O2	O1	T6	P3	P4	T5	T4	T3	C4	C3	F4	F3
20141126_1025			NE	FZA	PZA	CZA	FZB	PZB	CZB	O2	O1	T6	P3	P4	T5	T4	T3	C4	C3	F4	F3
20160413_1524			E	FZA	PZA	CZA	FZB	PZB	CZB	O2	O1	T6	P3	P4	T5	T4	T3	C4	C3	F4	F3

Table 2.1. TMS studies rejected for the quantitative analysis. The rows indicate the subjects and the columns the stimulation channels. The cells coloured in red indicate the stimulation channels with missing data, and the cells coloured in magenta highlight the excessively artefactual channels.

CHAPTER 3. RESULTS

C.3.1. Demography

Sixty-two patients and ten controls were recruited for the study. Of the original 62 recruited patients, two were withdrawn from the study after the final clinical classification was verified by Professor Koutroumanidis. One of the patients presented a superadded neuropsychiatric disorder (20141022_1013), and the other abandoned the follow-up appointment before a sound clinical diagnosis was established (20140915_1535). Of the final 60 patients who comprise our cohort for the study, twenty-seven out of the sixty were diagnosed as epileptic and thirty-three as non-epileptic based on clinical criteria. The median age in epilepsy and non-epilepsy patients was thirty-one and thirty years, respectively. Although not statistically significant, the gender ratio was slightly skewed towards females in the epilepsy group (11:16) but was balanced in non-epilepsy (17:16) and control groups (5:5).

Of the total twenty-seven subjects included in the epilepsy group, sixteen patients were diagnosed with genetic generalised epilepsy (GGE), which included two cases of juvenile myoclonic epilepsy (JME), two of juvenile absence epilepsy (JAE), two of epilepsy with eyelid myoclonia and absence (ELMA), six of genetic generalised epilepsy with generalised tonic-clonic seizures only (GGE-GTCS) and one of idiopathic generalised epilepsy with phantom absences (GGE-PA). In three patients, no GGE subsyndrome could be specified. Eleven patients were diagnosed with focal epilepsy (FE), which included four cases of temporal lobe epilepsy (TLE), four of frontal lobe epilepsy (FLE), two of frontotemporal (F-T) epilepsy (one of them presented bilateral foci) and one case with lateralising but non-localising focal epilepsy (F-NL) (Figure 3.1).

	Epilepsy (n=27)	Non-Epilepsy (n=33)
Age	17 - 51 (31)	16 - 69 (30)
Gender (M: F)	11 : 16	17 : 16
Medication (Y: N)	16 : 11	6 : 27
Motor threshold	40 - 85 (56)	40 - 83 (64)
MRI (N: A: No)	18 : 7 : 2	27 : 1 : 5
Baseline EEG epileptic activity (Y: N)	16 : 11	2 : 31
Complete: Incomplete	19 : 8	24 : 9

Table 3.1. The table illustrates the following characteristics in the sample of 60 patients used for the visual analyses of the RRs. Age, age range and median; Gender, gender ratio; Medication, use of antiepileptic medication at the time of the TMS study; Motor threshold, TMS resting motor threshold, range and median; MRI, MRI data (N, normal; A, abnormal; No, no performed); Baseline EEG epileptic activity, presence of epileptiform dischargers in the baseline EEG performed prior to the TMS-EEG study (Y, yes; N, no), Complete: Incomplete, ratio of patients who had a complete TMS-EEG study (the entire TMS-EEG study protocol was performed) or an incomplete TMS-EEG study (some stimulation areas of the TMS-EEG study protocol were not fully accomplished).

All focal epilepsy (FE) patients underwent brain magnetic resonance imaging (MRI). Seven of these eleven patients had an abnormal MRI: a hippocampal asymmetry with increased signal in the dentate gyrus was detected in one of them; in two cases, gliosis was detected (inferior frontal and temporal/ frontal gliosis), a frontal cortically-based mass, a frontal lobe tumour, cortical dysplasia and a post-traumatic lesion. No abnormalities were found in the remaining patients. MRI scan was performed in fourteen out of the sixteen patients with generalised epilepsy, and the MRI was normal in all these fourteen cases.

Of the total thirty-three non-epileptic subjects, twelve were classified as psychogenic non-epileptic seizures (PNES), fifteen as vasovagal syncope (VVS), three as acute symptomatic seizures (AS) and the remaining three as non-epileptic but the final

diagnoses remained uncertain. Twenty-eight out of the thirty-three non-epileptic subjects had an MRI scan that was abnormal in one case. This was a patient with VVS showing ischemic brain damage and bilateral frontal encephalomalacia on MRI (Table 3.1, Figure 3.1).

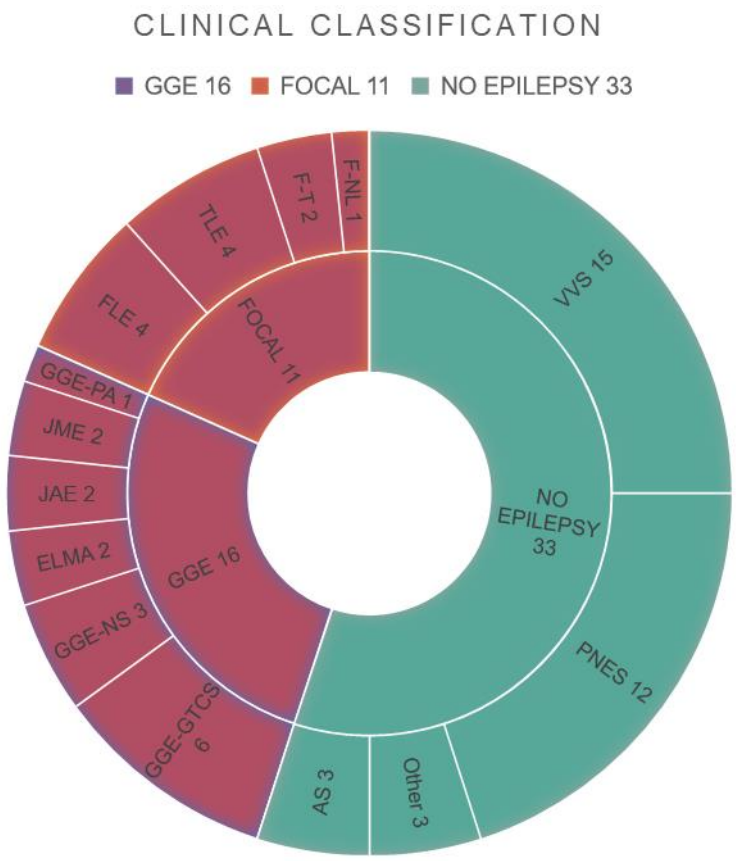


Figure 3.1. Clinical classification. The graph shows the clinical classification profile distribution in the total sample with 60 subjects used for the visual analyses of the RRs. GGE, genetic generalised epilepsy; JME, juvenile myoclonic epilepsy; JAE, juvenile absence epilepsy; ELMA, epilepsy with eyelid myoclonia and absence; GGE-GTCS, genetic generalised epilepsy with generalised tonic-clonic seizures only; GGE-PA, genetic generalised epilepsy with phantom absences; NS, no specified subsyndrome; FE, focal epilepsy; TLE, temporal lobe epilepsy; FLE, frontal lobe epilepsy; F-T, frontotemporal epilepsy; F-NL, lateralising but non-localising focal epilepsy; AS, acute symptomatic; PNES, psychogenic non-epileptic seizures; VVS, vasovagal syncope

C.3.2. Motor Threshold (MT)

The mean MT values in our sample did not differ between epileptic and non-epileptic patients (*t* (58) =1.76, p=0.08). The result is not significant at $p < 0.05$. Removing the patients under AEDs treatment from the analysis does have an impact, and there was a statistically significant difference in the MT between untreated epileptic and non-epileptic patients. The 11-drug naïve epileptic participants (M=54.54, SD=11.76) compared to the 27 participants in the drug naïve non epileptic group (M=62.86, SD=11.41) demonstrated lower MT values, *t* (38)= -2.04, p=0.05 (Figure 3.2B, C).

C.3.3. TMS-EEG recording protocol optimisation

After the assessment of a small cohort comprised of ten volunteers and ten patients, the TMS stimulation over the Fp1, Fp2, F7 and F8 positions were found to be particularly uncomfortable by the studied subjects, resulting in TMS-EEG recordings marred by profuse muscle artefacts which failed to provide additional relevant data. Therefore, these stimulation sites were removed from the final TMS stimulation protocol.

C.3.4. Report of unexpected side effects of TMS-EEG

No unexpected side effects of TMS-EEG were observed in this study. Some patients experienced minor discomfort due to the TMS device directly stimulating the contraction of muscles around the scalp and face. Usually, repositioning the TMS coil abolished the problem. No further modifications in the location of stimulation sites were required.

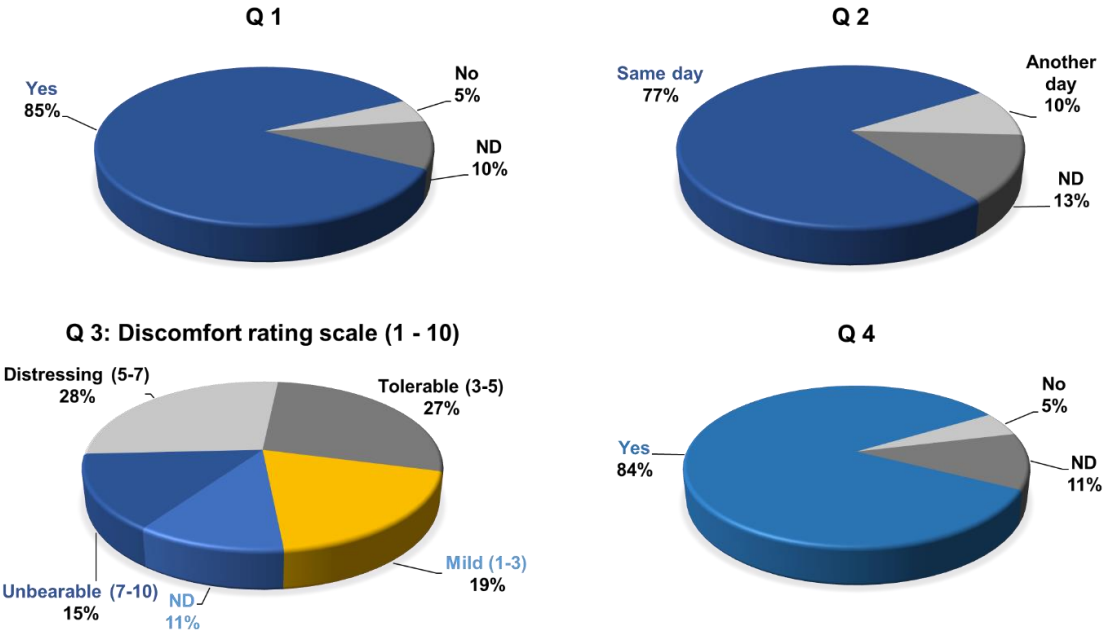
46% of the patients found the test tolerable, but the rest complained of discomfort, particularly when the TMS stimulation was near the temporal areas and in 17 cases

the TMS stimulation over these areas had to be abandoned. Overall, 85% of the patients would be willing to do the TMS study again if it was proven to be clinically useful for the management of their condition. In contrast, the EEG is painless, with minimum discomfort even during the activation techniques, and all the patients in the sample complied with the baseline EEG.

C.3.5. Evaluation of the patient acceptability for the duration of the final TMS-EEG protocol.

A brief feedback questionnaire was completed by all the patients after the recording in order to establish the most suitable TMS-EEG protocol in terms of patient comfort and time optimisation without compromising the reliability of inducing TMS-EEG responses based on the experience of the studied subjects. The results of the questionnaire are conveyed in the figure below.

A.)



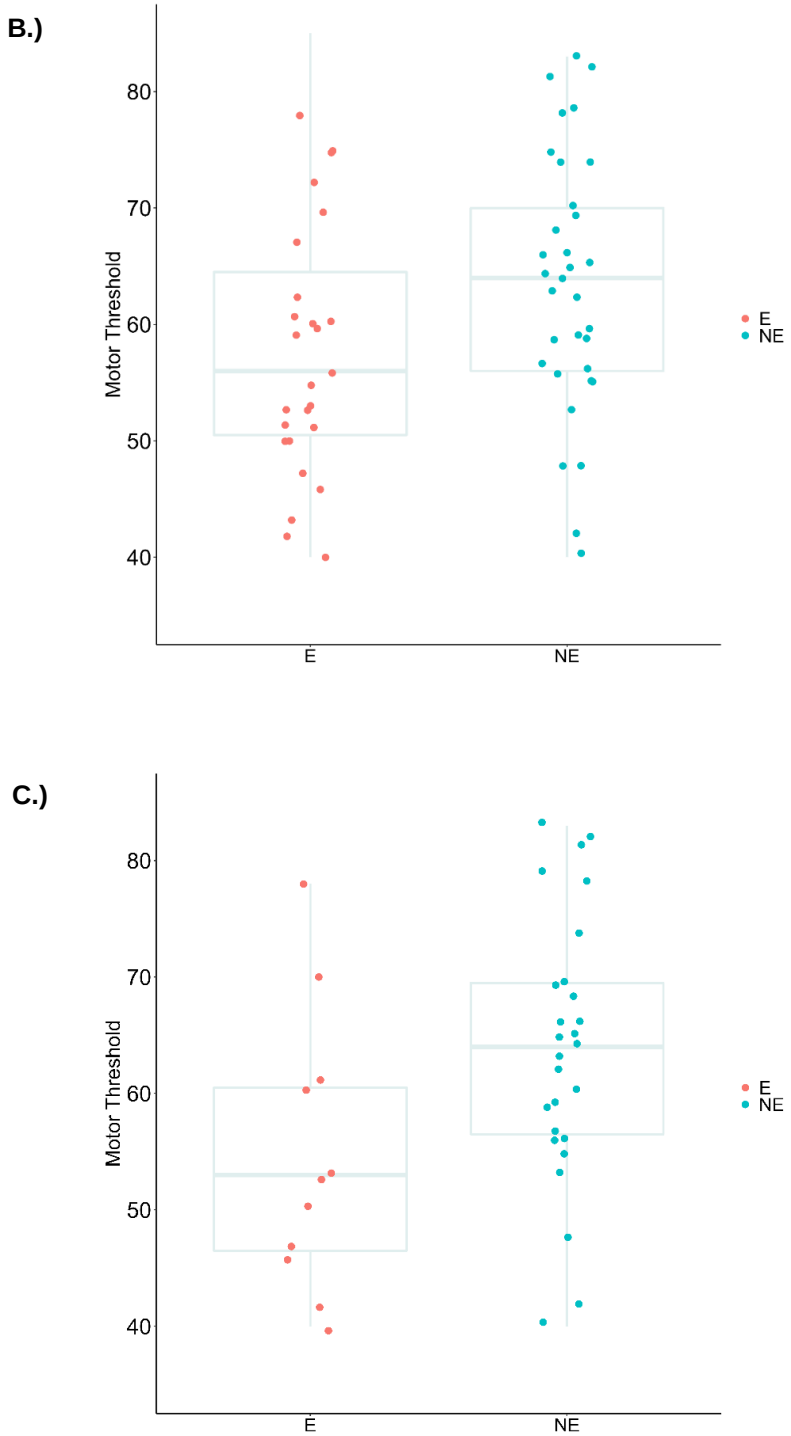


Figure 3.2. Feedback questionnaire and motor threshold values. **A.)** Feedback questionnaire results **Q1**, Question 1: Would you do the TMS study again if it was proven to be clinically useful for the management of your condition? 85% of the patients answered yes, 5% no. **Q2**, Question 2: Would you prefer to come for the TMS-EEG test as a separate appointment on a different day, or would you rather have both the routine baseline EEG and

TMS-EEG test on the same day? 77% of the patients did prefer to have the baseline-EEG and TMS-EEG on the same day, 10% on separate appointments. **Q3**, Question 3: On a visual analogue scale (VAS) from 1 to 10, 10 being the worst, the subjects were asked to rate the level of discomfort during the TMS study. For illustration purposes, the level of discomfort was classified as mild (VAS:1-3), tolerable (VAS: 3-5), distressing (VAS: 5-7) and unbearable (7-10) being 19%, 27%, 28% and 15% respectively the percentages of subjects ascribed to each subgroup. **Q4**, Question 4: Was the test procedure properly explained to you? If not, how can we improve it? 84% of the patients answered yes, 5% no. The most frequent complaint from the patients who answered "no" was not receiving the information leaflet in the post. ND indicates that no data was collected. **B.)** Motor threshold values of a sample of 58 patients (E, 27 subjects with epilepsy, NE, 31 subjects in the no-epilepsy group. **C.)** Motor threshold values of a sample of 38 drug naïve patients (E, 11 subjects with epilepsy, NE, 27 subjects in the no-epilepsy group

C.3.6. Qualitative interpretation of TMS evoked EEG responses

C.3.6.1. Interictal epileptiform discharges (IEDs) in the TMS-EEG and sleep-deprived EEG (baseline EEG) studies.

The TMS-EEG and sleep-deprived EEG (baseline EEG) recordings were visually inspected, and the number of spikes, sharp waves and interictal discharges (IEDs) were recorded. The number, duration and location of the IEDs in the baseline EEG and TMS-EEG studies were quantified, and a comparison was then made between the TMS-EEG findings and the baseline EEG.

Sixteen out of the sixty patients analysed (27 epilepsy, 33 no epilepsy) presented interictal epileptiform discharges in the baseline EEG. All these subjects had a final clinical classification of epilepsy; therefore, 16 out of the 27 patients with epilepsy had IEDs, and none of the patients who were diagnosed as not epilepsy had overt IEDs.

1) Comparison of the number of IEDs between TMS-EEG and baseline EEG in the sixteen epileptic subjects' cohort. In 4 out of 16 patients, there was an increase with

TMS in the number of IEDs per minute of recording of more than 50%. In 6 out of 16, there was a decrease of more than 50%, and in 6, there were no differences of more than 50%. There is no statistically significant difference in the number of IEDs recorded in the TMS-EEG in comparison to the baseline EEG performed prior to the TMS testing (paired test, p=0.34) (Figure 3.3A).

2) Comparison of all the IEDs' total time-duration divided per minutes of recording between TMS-EEG and baseline EEG. In 4 out of 16 patients, there was an increase with TMS in the IEDs' time duration per minute of more than 50%. In 5 out of 16, there was a decrease of more than 50%, and in 7, there were no differences of more than 50%. There is no statistically significant difference in the IEDs duration per minute (paired t-test, p=0.26) between TMS-EEG and baseline EEG (Figure 3.3B).

3) Comparison of the median duration of the IEDs between TMS-EEG and baseline EEG. In 2 out of 16 patients, there was an increase with TMS in the IEDs' median duration of more than 50%. In 2 out of 16, there was a decrease of more than 50%, and in 12, there were no differences of more than 50%. There is no statistically significant difference in the median duration of the discharges (paired t-test p=0.5) between TMS and baseline studies (Figure 3.3C).

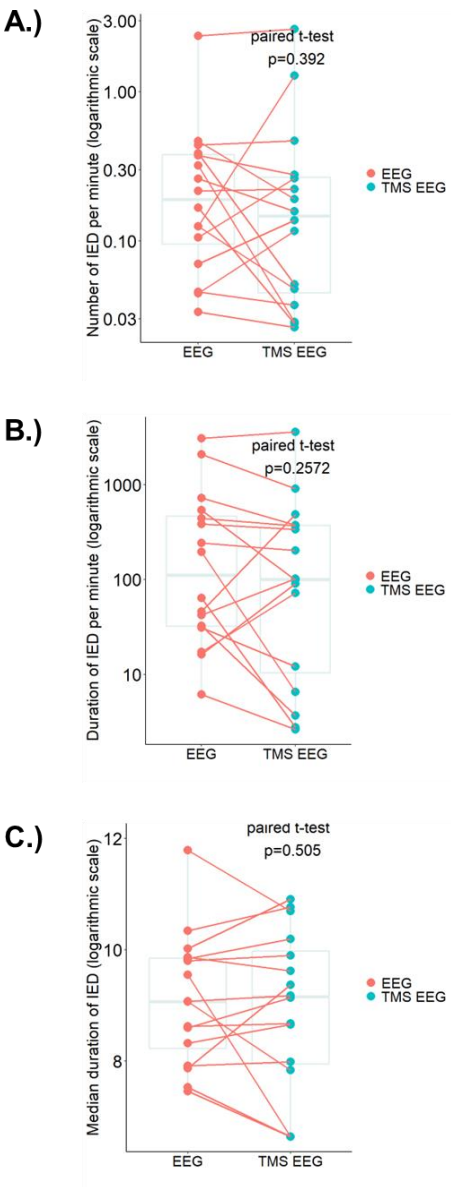


Figure 3.3. Comparison of the number, sum duration and median duration of the interictal epileptiform discharges (IEDs) between baseline EEG and TMS-EEG in each of the 16 epileptic subjects. A. The number of IEDs per minute of recording in baseline EEG and TMS-EEG. **B.** Sum duration of all the IEDs (milliseconds) divided per minute of recording in baseline EEG and TMS-EEG. **C.** Median duration (milliseconds) of the IEDs in baseline EEG and TMS-EEG. In each subject, the number, sum duration and median duration values are compared between the baseline EEG (red dots) and the TMS-EEG (blue dots) by means of the matched paired comparison performed by paired t-test.

C.3.6.2. Nonspecific discharges (NSDs) in the TMS-EEG and baseline EEG studies.

Ten out of the sixty patients had nonspecific interictal discharges (NSDs) of uncertain clinical significance in the baseline EEG, which were not reported as clearly epileptiform. Four out of these ten patients were classified as epileptic patients and six as non-epileptic patients.

1) Comparison of the number of NSDs between TMS-EEG and baseline EEG: In two (2 epilepsy, 0 no-epilepsy) out of the ten patients, there was an increase with TMS of >50% in the number of NSDs per minute of recording. In six (1 epilepsy, 5 no-epilepsy), there was a decrease of more than 50%, and in two (1 epilepsy, 1 no-epilepsy), there were no differences of more than 50% (Figure 3.4A-C). Statistically, the number of nonspecific discharges did not differ between the TMS-EEG and baseline EEG studies (paired t-test, p=0.37, Figure 3.4A). Once the group is dichotomised based on the clinical category, the baseline EEG/ TMS-EEG difference in the number of the NSDs in the non-epilepsy subgroup interestingly shows a tendency to a reduction in number in the TMS study, reaching statistical significance (paired t-test, p=0.03, Figure 3.4C). No significant difference was noted in the epilepsy subgroup (paired t-test, p=0.58, Figure 3.4B). There are not statistically significant differences between epileptic and non-epileptic subgroups in the differential number of NSDs between baseline EEG and TMS EEG (independent sample t-test, p=0.13, Figure 3.4D); however, the number of patients in the groups is very small, and no reliable inference can be made.

2) Comparison of all the NSDs total time-duration divided per minutes of recording between TMS-EEG and baseline EEG: In two (2 epilepsy, 0 no-epilepsy) out of 10

patients, there was an increase with TMS of the NSDs time duration per minute of more than 50%, in five (1 epilepsy, 4 no-epilepsy) out of 10 there was a decrease of more than 50%, and in three (1 epilepsy, 2 no-epilepsy) there were no differences of more than 50% (Figure 3.5A-C). Statistically, the sum time-duration of the runs of NSDs did not differ between baseline EEG and TMS-EEG studies (paired t-test, p=0.09, Figure 3.5A). Once the group is dichotomised based on the clinical category, the baseline EEG/ TMS-EEG differences in the sum duration of the NSDs becomes statistically significant in the non-epileptic subgroup only, showing a reduction in duration per minute in the TMS study (paired t-test, p=0.03, Figure 3.5C); however, the number of patients in the groups is small. No difference was noted in the epilepsy subgroup (paired t-test, p=0.62, Figure 3.5B). The sum duration subtraction values did not show statistically significant differences between epilepsy/ no epilepsy groups (independent sample t-test, p=0.2, Figure 3.5D).

3) Comparison of the median duration of the NSDs between TMS-EEG and baseline EEG. In none of the ten patients (4 epilepsy /6 no-epilepsy) was an increase with TMS in the NSDs median duration of more than 50%. In three (1 epilepsy, 2 no-epilepsy) out of 10, there was a decrease of more than 50%, and in seven (3 epilepsy, 4 no epilepsy), there were no differences of more than 50% (Figure 3.6A-C). The median duration of the NSDs shows a statistically significant reduction in the TMS study (paired t-test, p=0.03, Figure 3.6A), being the non-epileptic group the major contributor with a tendency to reduction in the TMS study no reaching statistical significance (paired t-test, p=0.09, Figure 3.6C); however, the number of patients in the subgroup is small. No difference was found between TMS-EEG and baseline EEG studies in the epilepsy group (paired t-test, p=0.29, Figure 3.6B). The median duration subtraction

values did not show statistically significant differences between epilepsy/ no-epilepsy groups (independent sample t-test, p=0.86, Figure 3.6D).

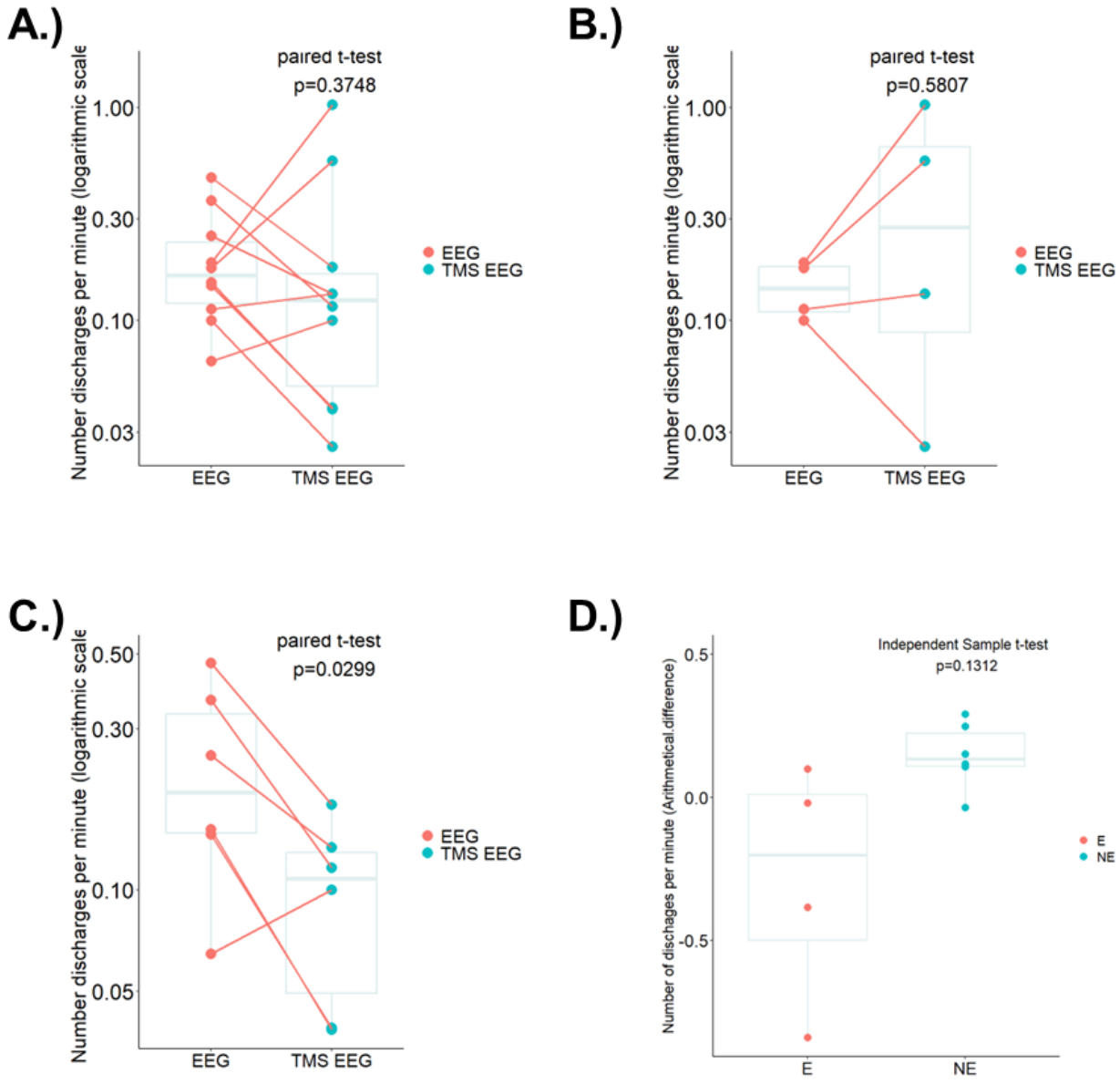


Figure 3.4. Comparison of the number of nonspecific discharges (NSDs) between baseline EEG and TMS-EEG in ten subjects. **A.** Number of NSDs per minute of recording in baseline EEG and TMS-EEG in ten subjects (four epilepsy, six non-epilepsy). **B.** Number of NSDs per minute of recording in baseline EEG and TMS-EEG in the epilepsy cohort (four subjects). **C.** Number of NSDs per minute of recording in baseline EEG and TMS-EEG in non-epilepsy cohort (six subjects). **D.** Arithmetical subtraction of the number of NSDs per minute of recording (TMS-EEG minus baseline EEG) showing no statistically significant difference between the epilepsy and no epilepsy groups.

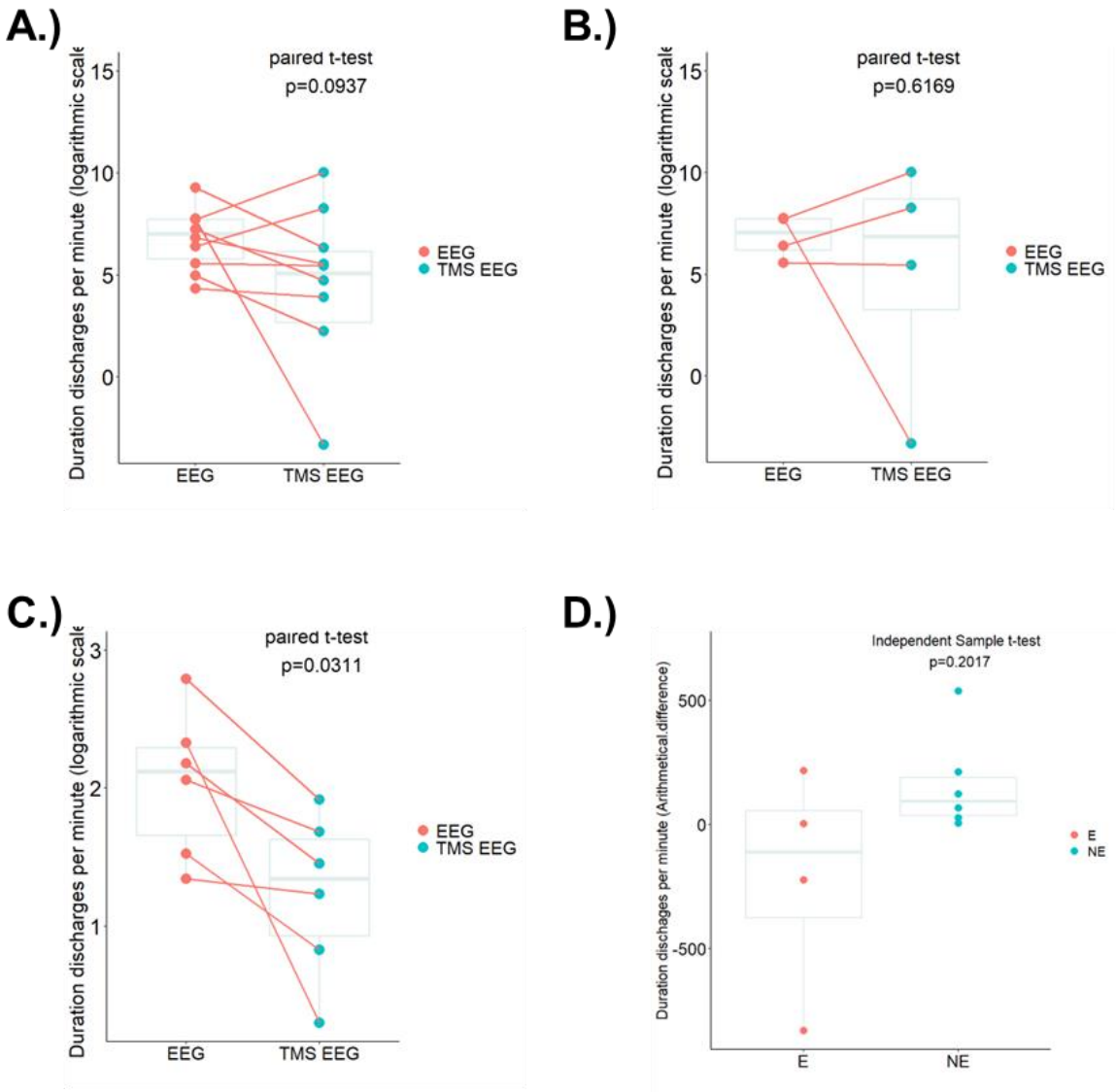


Figure 3.5. Comparison in the sum duration of the nonspecific discharges (NSDs) between routine EEG and TMS-EEG. **A.** Sum duration of NSDs per minute of recording in routine EEG and TMS-EEG in ten subjects (four epilepsy, six non-epilepsy). **B.** Sum duration of NSDs per minute of recording in routine EEG and TMS-EEG in the four subjects' epilepsy cohort **C.** Sum duration of NSDs per minute of recording in the routine EEG and the TMS-EEG in the six subjects' non-epilepsy cohort. **D.** Arithmetical difference of the sum duration of NSDs per minute of recording between the routine EEG and the TMS-EEG in the epilepsy and non-epilepsy groups.

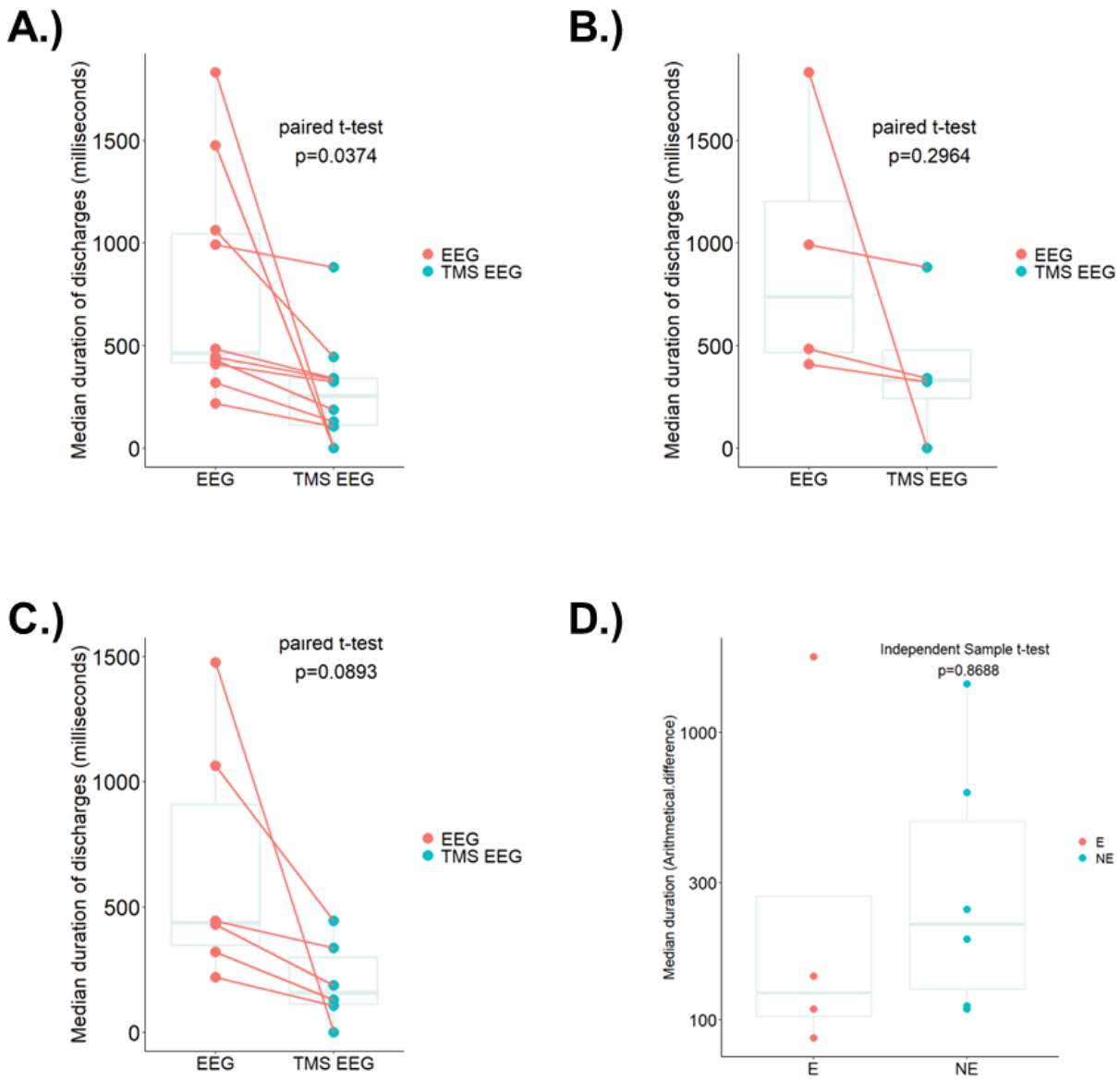


Figure 3.6. Comparison of the median duration of the nonspecific discharges (NSDs) between baseline EEG and TMS-EEG. **A.** Median duration of NSDs showing statistically significant reduction in the TMS-EEG in the full cohort of ten subjects (four epilepsy, six non-epilepsy) who presented NSDs. **B.** Median duration of the NSDs in the baseline EEG and TMS-EEG in the four subjects' epilepsy cohort. No significant difference is seen in the median duration between the baseline EEG and the TMS-EEG. **C.** Median duration of the NSDs in the baseline EEG and the TMS-EEG in the six subjects' non-epilepsy cohort. No statistically significant reduction in the median duration is seen in the TMS-EEG study. **D.** Arithmetical subtraction of the median duration of the NSDs, TMS-EEG minus baseline EEG, showing no statistically significant difference between the epilepsy and the non-epilepsy groups.

C.3.6.3. TMS EEG evoked late responses

A qualitative analysis of the two different types of late responses: delayed responses (DRs) and repetitive responses (RRs), was made. The relation of the late responses to the final clinical classification was assessed.

TMS induced delayed response (DRs)

DRs were visually assessed in my sample (Figure 3.7). Waveforms resembling IEDs seen between >100 msec and < 1-second post-TMS stimulation were classified as DRs related to TMS stimulation if the two-tailed sign test used to compare IEDs 1 second before and after TMS stimulation was significant.

DRs were found in 4 out of 16 patients with GGE (20140414_1238, 20140428_1105, 20140602_1039, 20140730_1051. These DRs occurred in patients with RRs present in the TMS-EEG and IEDs (3 cases) or NSDs (1 case) seen in the TMS-EEG and the previous baseline EEG. In one patient, 20140414_1238, the first DRs appear immediately after the TMS pulse resembling the zero time-lag evoked discharges described by the Kimiskidis' group. These DRs were followed by other TMS evoked DRs (Figure 3.8). This patient also had spontaneous IEDs in the baseline EEG and additional spontaneous IEDs in the TMS-EEG not induced by the TMS pulses.

The DRs appear only in patients with GGE, and they were not seen in any of the 11 patients with focal epilepsy, nor were DRs recorded in the VVS or PNES participants. Therefore, DRs appear in four out of twenty-seven subjects clinically classified as epileptic, giving a sensitivity of 14.81% and specificity of 100%.

The presence of DRs had a statistically significant association with the presence of epilepsy (Fisher's exact test, p=0.036).

In one of the patients with GGE and DRs (20140730_1051), the baseline was normal, with NSDs considered inconclusive in the final report (Figure 3.9). The other patients also had spontaneous IEDs in the baseline EEG and the TMS-EEG (Figure 3.10).

Dubious DRs: In one case, 20140811_1218, the only remarkable finding seen during the TMS-EEG study was a single burst of ill-defined sharply contoured slow waves. This feature, morphologically resembling NSDs, did not fulfil the criteria of DRs or evoked IEDs, and the TMS-EEG was finally classified as normal. This patient had a normal baseline EEG and a final clinical diagnosis of focal epilepsy (temporal lobe epilepsy) (Figure 3.11).

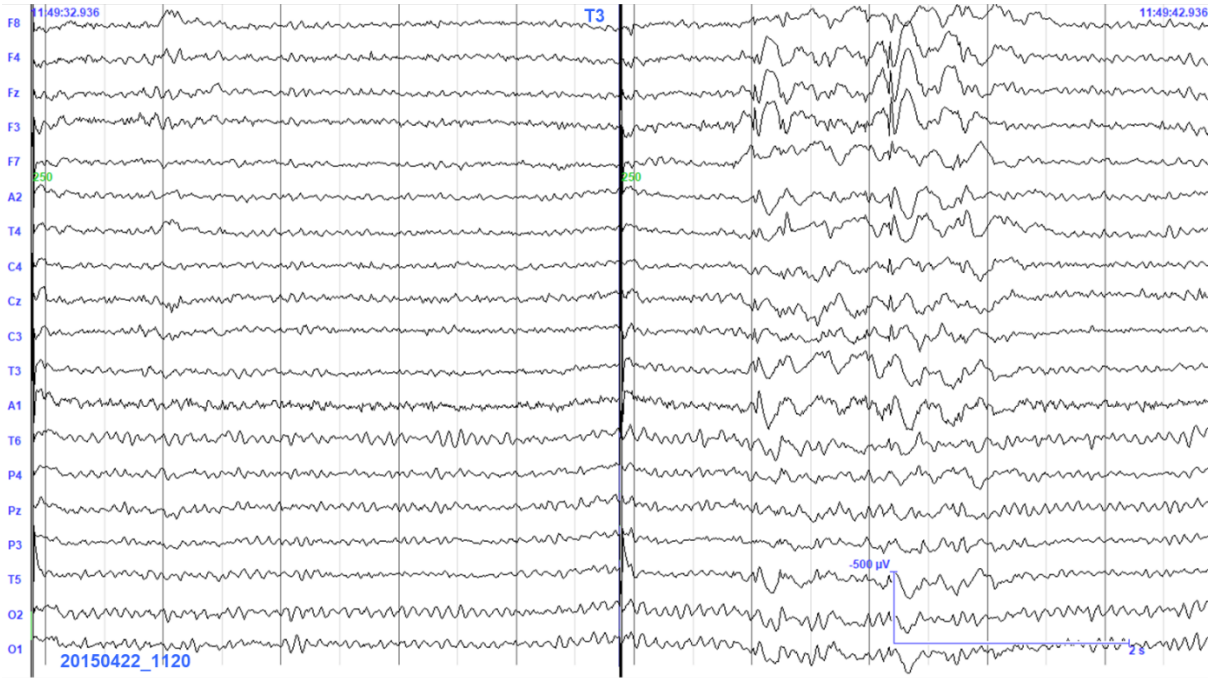


Figure 3.7. An illustrative example of delayed responses (DRs). The DRs appear within one second of single-pulse TMS stimulation in a GGE patient.

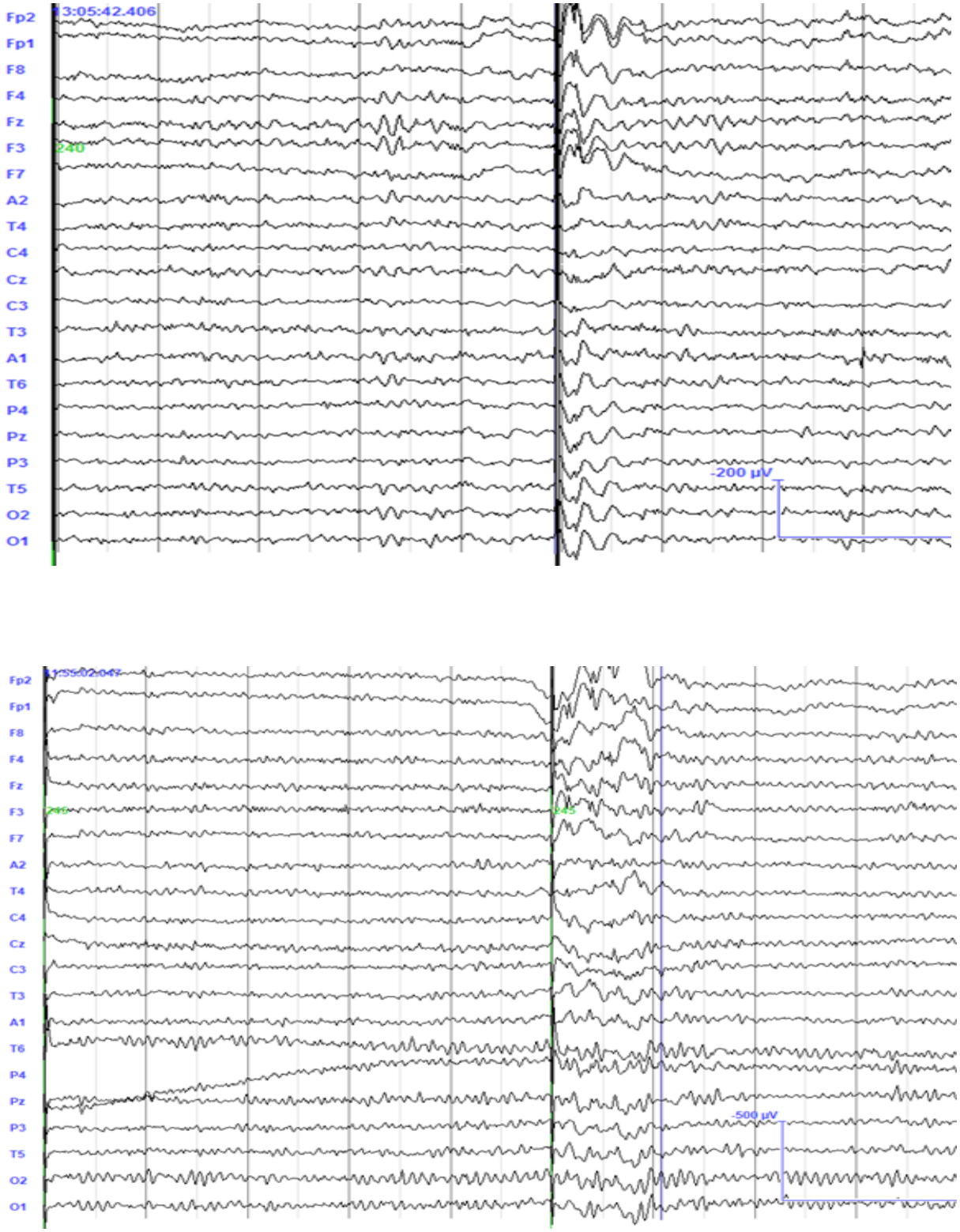


Figure 3.8. TMS-induced delayed responses (DRs) with single pulse stimulation in a patient with GGE. The first spike is seen on the descending slope of the early response (N100 wave).

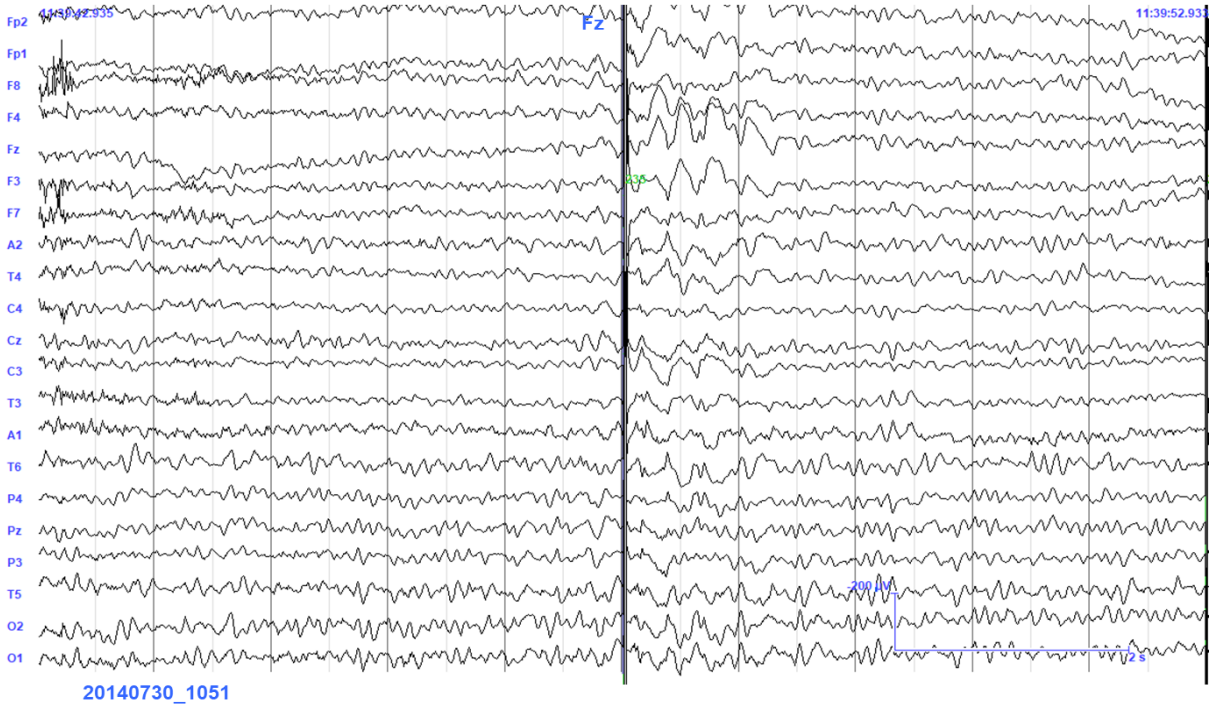


Figure 3.9. Example of delayed responses (DRs). DRs were seen within 100 milliseconds of a single pulse TMS stimulation over Fz applied in patient 20140730_1051.

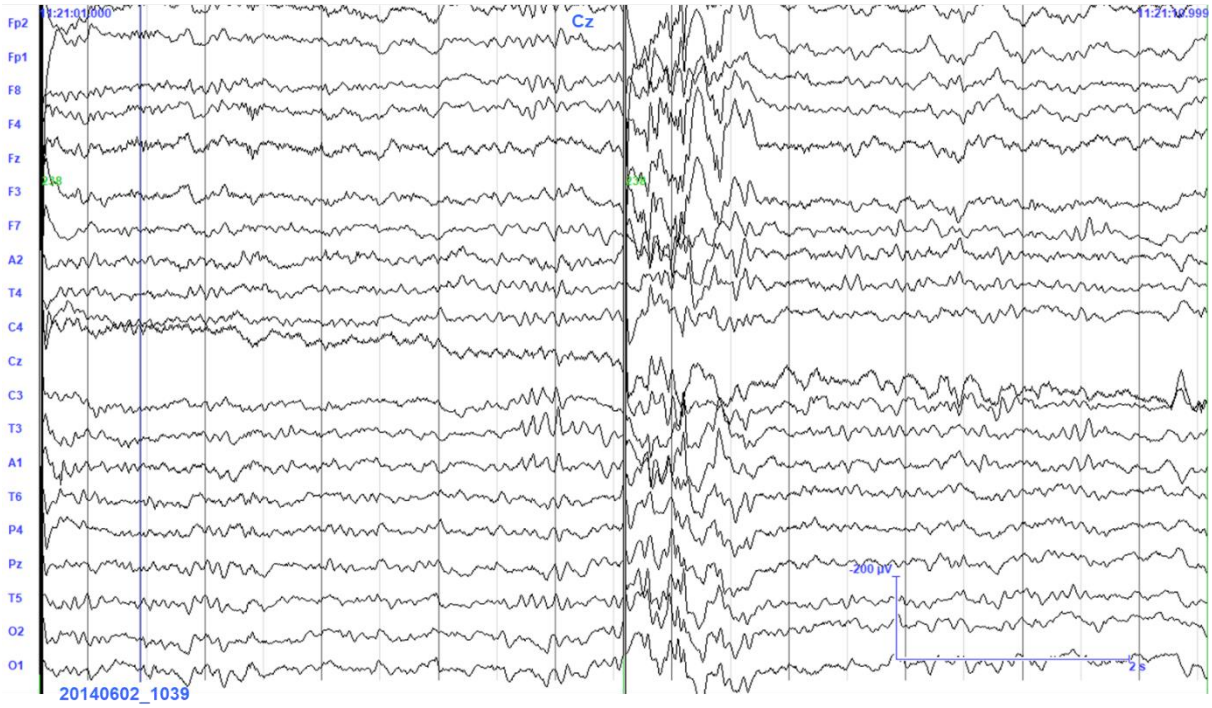


Figure 3.10. Example of delayed responses (DRs). DRs recorded in patient 20140602_1039.

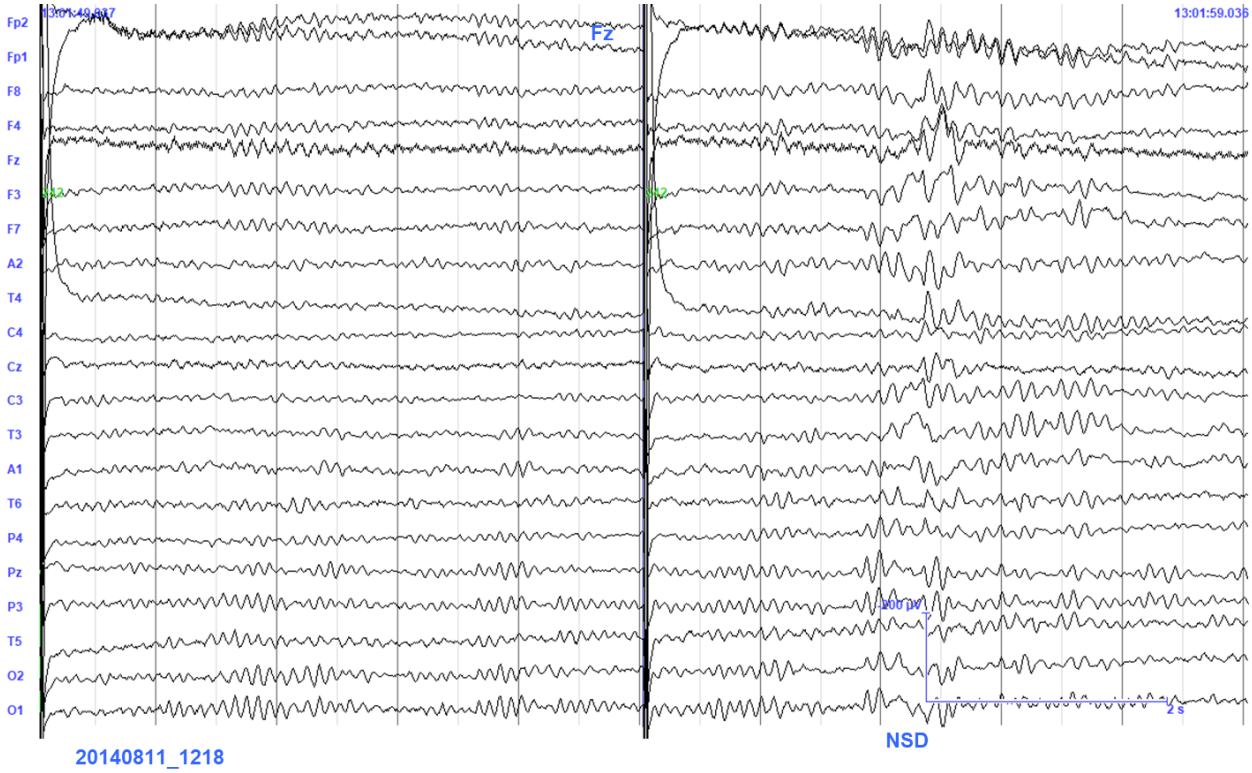


Figure 3.11. An illustrative example of incidental non-specific discharges (NSDs). NSDs were seen in patient 20140811_1218.

TMS induced repetitive responses (RRs)

RRs are defined as new-onset repetitive rhythms emerging in three or more recording channels following TMS stimulation at any given stimulation channel (without a contralateral counterpart), and it is hypothesised that these features (hereafter called abnormal TMS-EEG repetitive responses) are associated with epilepsy. This definition criteria of RRs was established by Valentin et. al. describing RRs in focal epilepsy (Valentin et al., 2008) and it was corroborated in my own experience in the preliminary analysis of the data showing that the cut off of 3 or more channels provides the best specificity value for RRs. Reducing the cut off value to 1 or 2 recording channels has a significant deleterious effect in the specificity: cut off 1 or more channels (sensitivity 89%, specificity 42%); cut off 2 or more channels (sensitivity 81%, specificity 48%); cut off 3 or more channels (sensitivity 63%, specificity 85%) (Table 3.3).

With two observers' agreement, the RRs appeared in 13 out of 27 epileptic patients, 4 of which had focal epilepsy and 9 GGE. RRs were also seen in three out of 33 non-epileptic patients. In 12 out of the 60 participants (7 epilepsy and 5 non-epilepsy), no agreement was found between the two observers (ML/AV), and the data was analysed by a third observer (GA).

After the input from the third observer, the RRs were seen in 17 out of 27 epileptic patients, 6 of which had focal epilepsy and 11 GGE. RRs were also seen in five out of 33 non-epileptic patients. Four out of the 17 GGE patients with RRs also had DRs provoked by TMS (201404_1238, 20140428_1105, 20140602_1039, 201408730_1051).

Summary of the positions of RRs recorded in TMS-EEG studies (Table 3.3).

Seventeen out of 27 epileptic subjects (6 FE, 11 GGE) had RRs. In 8 out of the 17 cases (4 FE, 4 GGE), the RRs appear upon stimulation in the midline channels exclusively (Fz, Cz, Pz); in 3 cases (GGE), the RRs were evoked upon stimulation in the midline and frontal channels, in two cases (GGE) with stimulation on frontal areas only, in two cases (1FE, 1GGE) with stimulation in C3 only, in one case (GGE) with stimulation in P3 only and in one case (FE) with stimulation of central midline and parasagittal areas (Cz, C4).

Five out of 33 non-epileptic subjects had RRs. In 3 out of the 5 cases, the RRs appear upon stimulation in the midline channels (Fz, Cz, Pz) exclusively; in 1 case, the RRs were evoked upon stimulation in the posterior areas; midline parietal, occipital and posterior temporal areas and in 1 case with stimulation on P3 only.

The RRs were mostly seen when stimulating extratemporal structures, apart from one exception: the participant (20160118_1033), who was the only patient showing RRs evoked by TMS stimulation over T5. This particular case was a non- epileptic patient with a final clinical diagnosis of VVS.

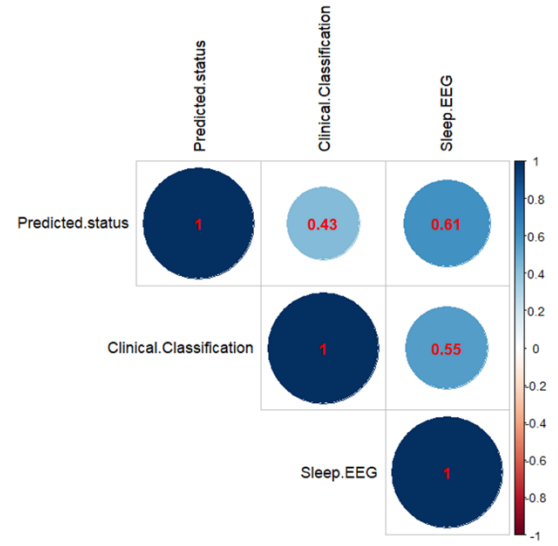
C.3.6.4. Correlation of TMS induced repetitive responses (RRs) in relation to clinical classification.

Based on the RRs definition, subjects in our sample with RRs were given a predicted TMS supported status of likely epileptic. This predicted RRs-TMS based status was evaluated against the final clinical diagnosis issued twelve months after the initial consultation in the First Seizure Clinic (Table 3.3).

In order to test the performance of the RRs-TMS based classification, the sensitivity and specificity of the RRs alone as a classifier were tested by comparing with the clinical information. Seventeen out of twenty-seven subjects clinically classified as epileptic and 5 out of thirty-three clinically classified as non-epileptic had an abnormal TMS study based on the presence of RRs, giving a sensitivity of 62.96% and specificity of 84.84% (Figure 3.12B). The recording of RRs alone was significantly associated with the presence of epilepsy (Fisher's exact test, p=0.0002). The significance of the association between the RRs and the clinical classification was evaluated by the Phi correlation coefficient giving the result of 0.43 (Figure 3.12A).

Of the 17 epileptic subjects with abnormal TMS based on the presence of RRs, six had focal epilepsy and 11 GGE. Therefore, 11 out of a total of 16 patients with GGE and six out of a total of 11 patients with FE had RRs in the TMS study giving a sensitivity of 68.75% and 54.54% for each epilepsy subgroup, respectively. The recording of RRs alone was significantly associated with the presence of epilepsy in the GGE subgroup (Fisher's exact test, p=0.0003) and FE subgroup (Fisher's exact test, p=0.0162).

A.)



B.)

Patient with epilepsy as confirmed by clinical diagnosis

		Positive	Negative	
TMS-EEG Classification (RR only)	Test Positive	17	5	22
	Test Negative	13	28	38
		27	33	
		Sensitivity (%)	Specificity (%)	
		62.96	84.84	

Figure 3.12. Correlation between repetitive responses (RRs) and clinical classification and baseline EEG. **A.** Phi correlation between the predictive status of the likelihood of epileptic trait based on the TMS-EEG results and the final clinical classification. In the correlation matrix heatmap, the size of the circle represents the significance of the pi value, and the colour denotes the correlation coefficient, being dark blue the highest correlation coefficient, value =1. **B.** Sensitivity and specificity test of the TMS evoked RRs to detect epilepsy, displaying the number of false positives, false negatives, true positives and true negatives when comparing the TMS-EEG results with the clinical classification.

C.3.6.5. Correlation of the TMS induced late responses (DR and/or RRs) with the clinical classification

The epileptic patients with abnormal TMS-EEG features had more recorded RRs than DRs (4/27 DRs and 17/27 RRs).

When considering the two different types of late responses together (DRs and/or RRs), a positive TMS-EEG test is seen in 17 out of the 27 epileptic patients (13/27 RRs alone, 4/27 RRs plus DRs) and in 5 out of 33 non-epileptic patients (RRs alone) (Table 3.2). Therefore, in this sample, the combined late responses (DRs and/or RRs) (Figure 3.13A) did not increase the sensitivity of the TMS-EEG test compared to the RRs alone (Figure 3.12B). The presence of late TMS-EEG responses (DRs and/ or RRs) was associated with the presence of epilepsy (Fisher's exact test, p=0.0002).

In two out of the four epilepsy patients with DRs plus RRs, there was an interobserver disagreement (AV/ML) for the presence of TMS evoked RRs. In these two cases (20140602_1039, 20140730_1051), the presence of DRs further supported the final third observer classification of abnormal TMS-EEG study. Therefore, the DRs may add additional support to the final classification of TMS-EEG in normal/ abnormal when the RRs are unclear.

C.3.6.6. Correlation of the TMS induced responses and clinical classification in patients with normal baseline EEG.

In our sample, eleven out of 42 participants with normal baseline EEG had a final clinical diagnosis of epilepsy (five FE, six GGE). Four out of the 11 epileptic patients with a normal baseline EEG (three GGE, one FE) had an abnormal TMS-EEG study; 3 show RRs alone and one RRs plus DRs. Therefore, in these four particular cases, the combined late responses (RRs and/or DRs) add certainty to the diagnosis of

epilepsy in comparison to the baseline EEG alone. The late responses correctly classified as epileptic 4 out of 11 (36%) patients with a false-negative baseline EEG (Figure 3.13D), increasing the sensitivity of the EEG to detect epilepsy from 59% with baseline EEG alone (Figure 3.13C) to 74% with TMS-EEG (late responses: RRs and/or DRs) added on to the routine EEG (Table 3.2) (Figure 3.13B). Among the 31 non-epileptic patients with normal baseline EEG, 3 had late responses (RRs alone). The late responses had no statistically significant association with the presence of epilepsy (Fisher's exact test, p=0.063) in patients with normal baseline EEG.

Focusing on the RRs, four out of these 11 epileptic patients with a normal baseline EEG presented an abnormal TMS-EEG based on RRs alone (20140609_1139, 20141201_1434, 20150615_1032, 20140730_1051, Figure 3.14). Therefore, the RRs alone did add valuable information to the diagnoses of epilepsy in 36% of patients with epilepsy with a normal baseline EEG study, increasing the sensitivity to detect epilepsy to 74% with RRs-TMS-EEG plus baseline EEG over the baseline EEG alone (59%) (Figure 3.13F). The rest of the patients with epilepsy with RRs had an abnormal baseline EEG, and the RRs did not add to the routine EEG in this scenario. The RRs had no statistically significant association with the presence of epilepsy (Fisher's exact test, p=0.063) in patients with normal baseline EEG.

Focusing on the DRs, one out of the four epileptic patients with a normal baseline EEG and abnormal TMS-EEG had DRs. So, the delayed TMS-EEG responses did add to the diagnoses over the baseline EEG alone in one case (20140730_1051) (Figure 3.9). This GGE patient had a normal baseline EEG, but the DRs were not the only abnormal feature in the TMS-EEG, as RRs were also recorded in the TMS-EEG. The rest of the epilepsy patients with DRs had an abnormal baseline EEG, and the DRs did not add to the baseline EEG in this scenario. Therefore, the DRs did add valuable

information to the diagnoses of epilepsy in 9% of epilepsy patients with a normal baseline EEG study, increasing the 59% sensitivity of the routine EEG alone to 63% (DRs-TMS add on to the routine EEG) (Figure 3.13.E). Among the 31 non-epilepsy patients with normal baseline EEG, none had DRs. The DRs had no statistically significant association with the presence of epilepsy (Fisher's exact test, p=0.263) in patients with normal baseline EEG.

In summary, relying exclusively on the late responses evoked by TMS to assign a diagnosis of epilepsy or no-epilepsy did increase the sensitivity of the test in comparison to the baseline EEG alone (63% RRs-TMS-EEG alone, 59% baseline EEG alone), but in some cases added a confounding factor that reduced the specificity as RRs were recorded in non-epileptic patients. The late responses correctly classified as epilepsy a 36% of patients with a false-negative baseline EEG. In patients with normal baseline EEG, the late responses had no statistically significant association with the presence of epilepsy (Fisher's exact test, p=0.063). However, when considering all the abnormal features provided by the TMS-EEG as an add-on to the routine EEG, considering the presence of IEDs and/or the presence of late responses (RR and/or DRs), the sensitivity increased from 59% with baseline EEG alone to 74% with TMS-EEG (late responses+ IEDs). The specificity was reduced from 94% with baseline EEG alone to 85% with add-on TMS-EEG.

DRs	RRs	TMS-EEG+IEDs	P.S. (LR=RRs and/or DRs)	P.S. (RRs-Only)	EEG-IEDs	Baseline EEG	C.C	N.P.
Y/N	Y	Y	E	E	Y	Ab	E	13
N	N	Y	NE	NE	Y	Ab	E	3
N	Y	N	E	E	N	N	E	3
Y	Y	N	E	E	N	N	E	1
N	N	N	NE	NE	N	N	E	7
N	Y	N	E	E	N	Ab	NE	2
N	Y	N	E	E	N	N	NE	3
N	N	N	NE	NE	N	N	NE	28

Table 3.2. Summary of abnormal TMS-EEG and baseline EEG findings. DRs, delayed responses; RRs, repetitive responses; IEDs, interictal epileptiform discharges; P.S., predicted status; LR, late responses (RRs and/or DRs); C.C., clinical classification; E, epilepsy; NE, no-epilepsy; N.P., number of patients; Yellow colour: C.C: E, predicted status: E, Baseline EEG: Normal; Orange colour: C.C: E, Baseline EEG: Abnormal, predicted status: NE; Light Orange colour: C.C: E, Baseline EEG: Normal, Predicted status: NE

A.)

Patient with epilepsy as confirmed by clinical diagnosis

		Positive	Negative	
TMS-EEG Classification (DR-RR)	Test Positive	17	5	22
	Test Negative	10	28	38
		27	33	
		Sensitivity (%)	Specificity (%)	
		62.96	84.84	

B.)

Patient with epilepsy as confirmed by clinical diagnosis

		Positive	Negative	
TMS-EEG Classification (DR-RR-IED)	Test Positive	20	5	25
	Test Negative	7	28	35
		27	33	
		Sensitivity (%)	Specificity (%)	
		74.07	84.84	

C.)

Patient with epilepsy as confirmed by clinical diagnosis

		Positive	Negative	
Baseline EEG Classification	Test Positive	16	2	18
	Test Negative	11	31	42
		27	33	
		Sensitivity (%)	Specificity (%)	
		59.25	93.93	

D.)

Patient with epilepsy as confirmed by clinical diagnosis and normal baseline EEG

		Positive	Negative	
TMS-EEG Classification (DR-RR)	Test Positive	4	3	7
	Test Negative	7	28	35
		11	31	
		Sensitivity (%)	Specificity (%)	
		36.36	90.32	

E.)

Patient with epilepsy as confirmed by clinical diagnosis

		Positive	Negative	
TMS-EEG Classification (DR+IED)	Test Positive	17	0	17
	Test Negative	10	33	43
		27	33	
		Sensitivity (%)	Specificity (%)	
		62.96	100	

F.)

Patient with epilepsy as confirmed by clinical diagnosis

		Positive	Negative	
TMS-EEG Classification (RR+IED)	Test Positive	20	5	25
	Test Negative	7	28	35
		27	33	
		Sensitivity (%)	Specificity (%)	
		74.07	84.84	

Figure 3.13. Contingency tables showing the sensitivity and specificity of the TMS-EEG test to detect epilepsy based on the presence of **A.)** TMS-EEG-late responses= DRs and/or RRs **B.)** TMS-EEG late responses and/or TMS-EEG interictal epileptiform discharges **C.)** the sensitivity and specificity of the baseline EEG test to detect epilepsy in this cohort. **D.)** the sensitivity and specificity of the TMS-EEG-late responses to detect epilepsy patients with a false negative baseline EEG. **E.)** the sensitivity and specificity of the TMS-DRs add on to the baseline EEG test to detect epilepsy in this cohort. **F.)** the sensitivity and specificity of the TMS-RRs add on to the baseline EEG test to detect epilepsy in this cohort.

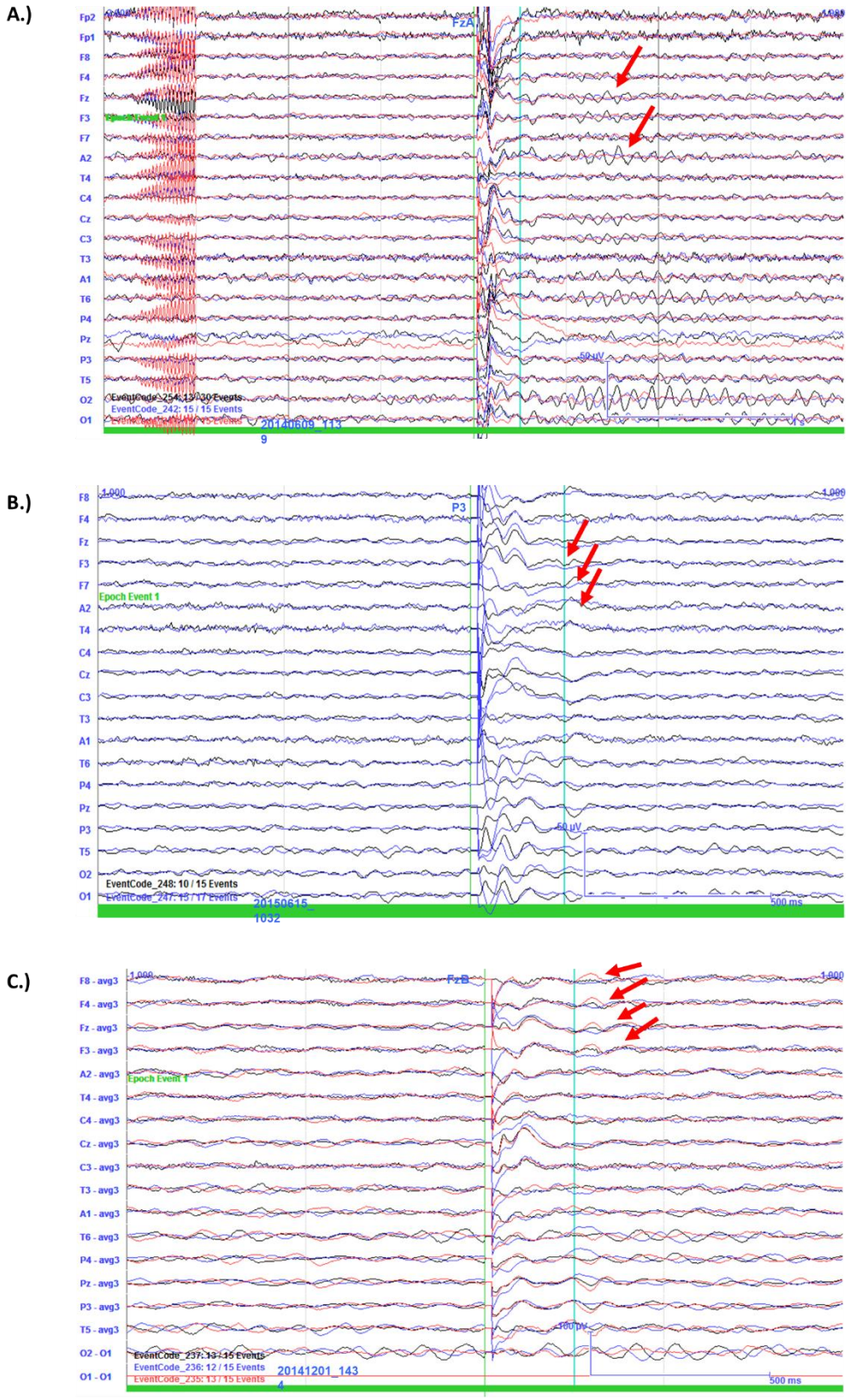


Figure 3.14. Abnormal TMS-EEG with repetitive responses (RRs) in the three epileptic patients with normal baseline EEG and final clinical classification of epilepsy. **A.)** 20140609_1139 **B.)** 20150615_1032 **C.)** 20141201_1434.

C.3.6.7. Reproducibility of the visual assessment of TMS induced RRs

The visual assessment of the RRs presents a certain degree of variability due to human factors when dealing with the complexity and elusive nature of the signals in our sample. Intra- observer and inter-observer variability studies were performed to address this matter. The TMS-EEG study was reviewed by two independent observers (Marian Lazaro: ML and Antonio Valentin: AV), looking for the presence of repetitive responses (RRs). The analysis was performed following the methodology described in the material and methods chapter, qualitative analysis section (C.2.2.4). Each observer scored the TMS-EEG responses twice to assess the intra-observer variability, and the inconsistencies between observer ML and observer AV were also measured.

To have a comprehensive appreciation of the intra-observer variability, the new onset repetitive rhythms found in all sixty subjects in two independent scorings by ML were compared using Cohen's Kappa Coefficient test (McHugh, 2012). The level of agreement was strong /almost perfect based on the overall Kappa value of 0.92 (Figure 3.15A-B) for ML/ML, sample=60. Subsequently, the same analysis was repeated in 33 randomly selected subjects with complete TMS study data set in order to evaluate the impact of incomplete data as a confounding factor in the analysis of the overall intra-observer reliability. The strength of agreement was then reduced to moderate with a kappa coefficient of 0.61 (Figure 3.15A) for ML/ML, sample=33.

One possible explanation for the disparity of the intra-observer agreement between the two different samples (ML/ML/60 vs ML./ML/33) and the reduction in the level of intra-observer agreement in the sample with complete TMS-EEG data (ML/ML/33), would be the impact of the missing data points. The missing values, which are easily

detectable upon visual inspection, may skew the agreement values to a higher score in the studies with an incomplete data set (ML/ML/60).

In the first stage, to calculate the level of intra-observer agreement (ML/ML), I studied the total sample of 60 subjects with both complete and incomplete data sets. Being the values with no data considered “0”, no data=”0”, the values with no new-onset repetitive rhythms (NORRs) present also considered “0”, no NORRs=”0” and the values with new-onset repetitive rhythms present (NORRs) considered 1, NORRs =”1” (Figure 3.15A). As the data points with no values were quite obvious to determine, the study gave the false impression of very high intra-observer agreement (ML/ML) in the n=60 sample. Therefore, a second analysis of the intra-observer variability (ML/ML) with a reduced sample of 33 studies with a complete data set was performed. This rendered a kappa value of 0.61 in keeping with a moderate level of intra-observe agreement for ML/ML/33.

The inter-observer agreement between two independent observers (ML/AV) was low, with a minimal level of agreement established by a low-value kappa coefficient (kappa=0.29, Figure 3.15A-B).

Upon observation of this high inter-observer variability (ML/AV), the intra-observer variability for AV/AV was also studied across a randomly selected sample of ten complete data set TMS-EEG studies. This study shows a low intra-observer agreement (kappa=0.22, Figure 3.15A-B).

A.)

ML/ML/60

	0	1	ND
0	19839	197	0
1	149	290	0
ND	0	0	2205

Kappa=0.92

ML/ML/33

	0	1
0	12084	118
1	98	174

Kappa=0.61

ML/AV/33

	0	1
0	12056	153
1	189	76

Kappa=0.29

AV/AV/10

	0	1
0	3587	70
1	96	27

Kappa=0.22

B.)

Value of Kappa	Level of agreement	% of data that are reliable
below 0	Weaker than expected by chance	
0	Expected by chance	
0 – 0.2	None	0 - 4 %
0.21 – 0.39	Minimal	4 - 15 %
0.40 – 0.59	Weak	15 - 35 %
0.60 – 0.79	Moderate	35 - 63 %
0.80 – 0.90	Strong	64 - 81 %
above 0.90	Almost perfect	82 - 100 %

C.)

ML/AV(60)	Value of Kappa	Level of agreement
CzA	0.35	Minimal
PzA	0.38	Minimal
FzA	0.31	Minimal
CzB	0.65	Moderate
PzB	0.31	Minimal
FzB	0.30	Minimal
C3	0.65	Moderate
C4	1	Almost perfect
P3	1	Almost perfect
P4	1	Almost perfect
O1	0.98	Almost perfect
O2	0.98	Almost perfect
T5	0.65	Moderate
T6	0.98	Almost perfect
F3	0.46	Weak
F4	0.95	Almost perfect
T3	0.96	Almost perfect
T4	0.96	Almost perfect

D.)

ML/AV/60

	0	1
0	30	7
1	5	15

Kappa=0.56

Figure 3.15. Intra-inter variability test (Kappa coefficient). A. Intra-observer variability test between two consecutive analyses of the new-onset repetitive rhythms (NORRs) in 60 TMS-EEG studies (ML/ML/60), 33 completed TMS-EEG studies (ML/ML/33), and 10 completed TMS-EEG studies (AV/AV/10) and inter-observer variability analysis between two independent observers ML/AV in a sample of 33 randomly selected completed studies (ML/AV/33). "1" refers to the presence of new-onset repetitive rhythms after TMS stimulation and "0" refers to no changes seen after TMS stimuli, and "ND" denotes no data available at a particular data point. **B.** Level of agreement between observations and observers based on Kappa values. **C.** Inter-observer variability (ML/AV/60) based on Kappa values for the presence of RRs in each stimulation channel (S.C.). **D.** The inter-observer (ML/AV/60) variability for the final TMS-EEG classification of 60 TMS-EEG studies based on RRs alone. "0" refers to a normal TMS-EEG study and "1" to an abnormal or positive TMS-EEG study.

To explicitly account for the complexity of the signals in a particular channel/stimulation point, the disagreement of the new-onset repetitive rhythms was subsequently scrutinised throughout each individual recording channel per simulation point. This data was presented in a heatmap showing the ML/ML intra-observer variability analysis (Kappa) for each of the 378 data points (21 recording channels per 18 stimulation points) on the selected 60/33 patients' samples (Figure 3.16A-B). The disagreement does not favour a particular stimulation point but tends to occur in the anterior recording channels (Fp1, Fp2, F3, F7, F4 and Fz) in contrast to temporal, parietal and posterior locations.

The AV/AV intra-observer disagreement occurs randomly across the different data points with no particular pattern or emphasis in any given location, stimulation point or recording channel (Figure 3.16D). Likewise, the inter-observer variability (ML/AV) does not seem to favour any particular pattern (Figure 3.16C).

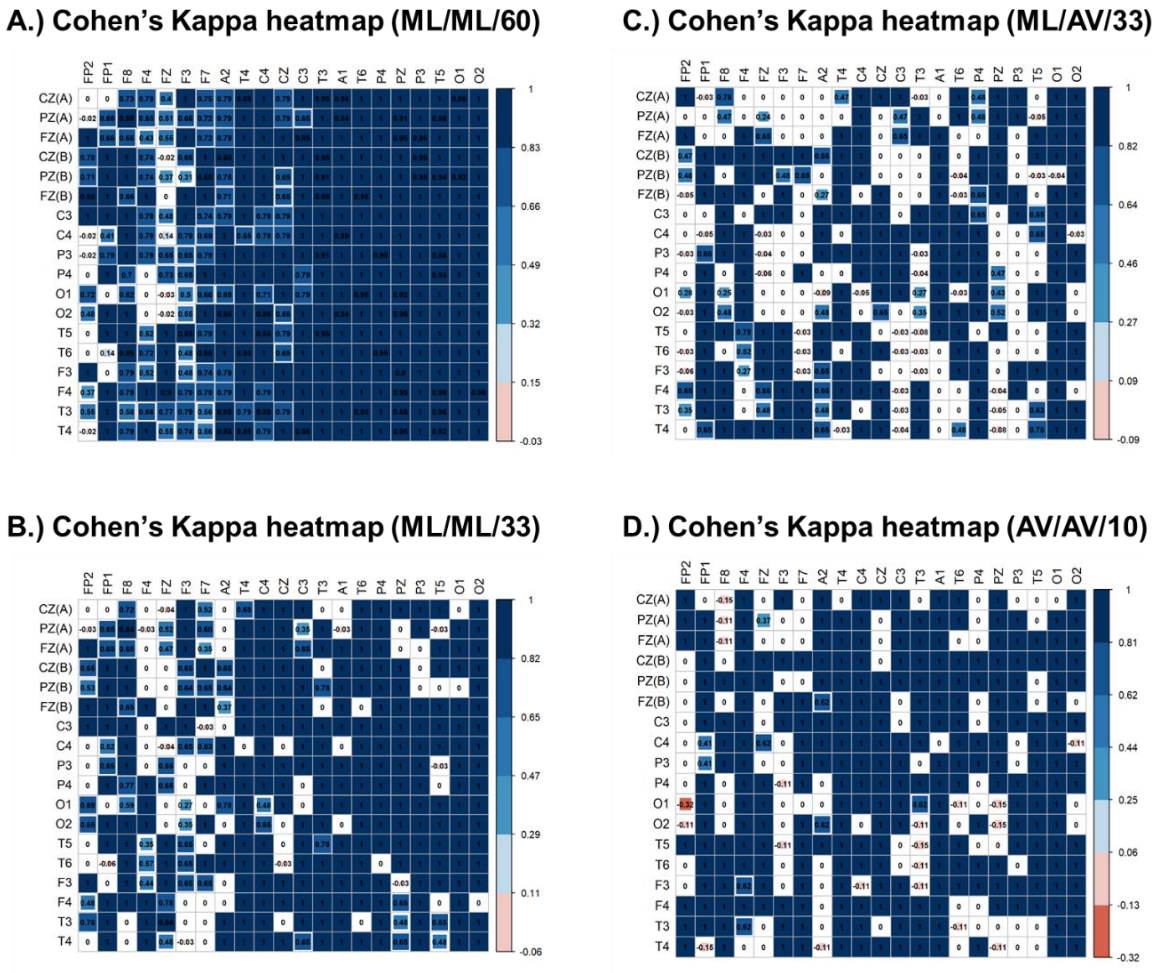


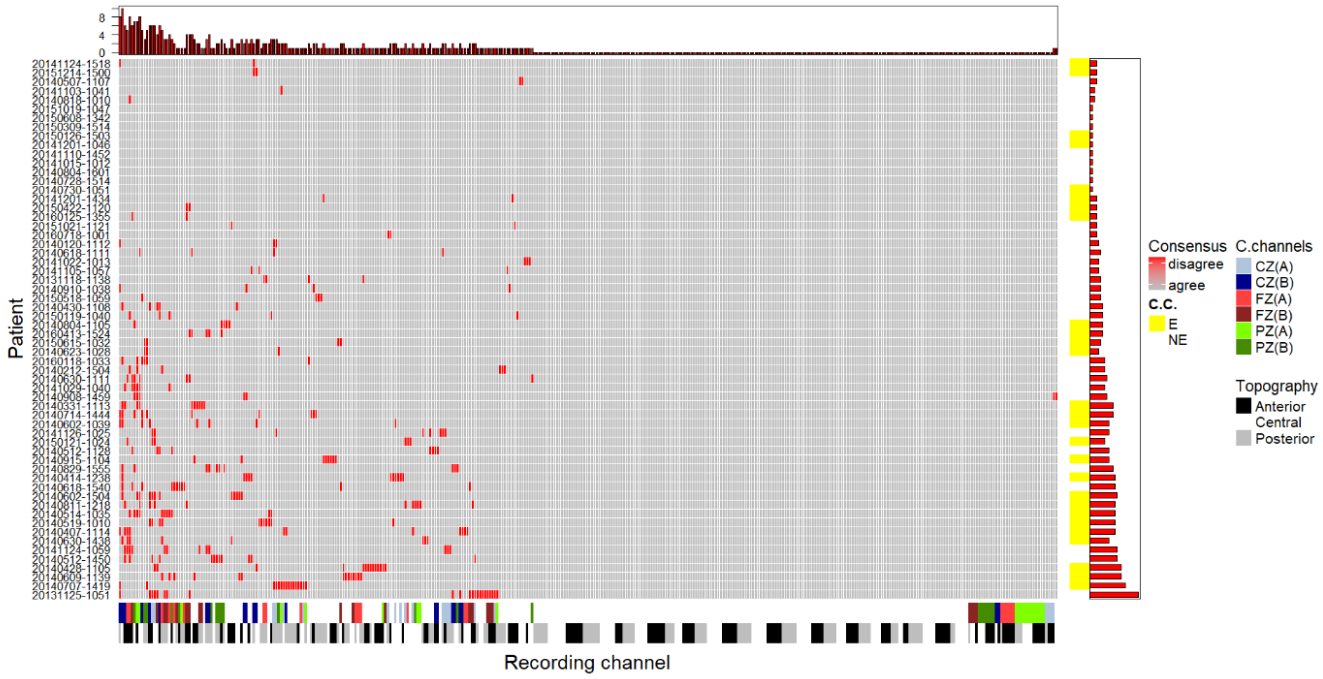
Figure 3.16. Cohen's Kappa heatmap for each recording channel at every stimulation area (data point) on selected patients. A. Intra-observer variability for observer ML (ML/ML) assed in each individual data point comprised of 18 stimulation areas recorded over 21 channels. The sample consists of 60 studies, out of which 43 have a complete dataset. **B.** Individual data point intra-observer variability for ML/ML in 33 subjects with a fully complete data set. **C.** Inter-observer variability between two independent observers ML and AV (ML/AV) for each individual data point on the same randomly selected sample of the 33 complete dataset studies. **D.** Individual data point intra-observer variability for observer AV (AV/AV) on ten randomly selected fully complete studies. In each heatmap figure, the X-axis represents the 21 recording channels, and the Y-axis displays the 18 stimulating points. Each square represents each of the 378 data points for the study. The number in each square is the Kappa coefficient for each data point, and the numerical value corresponds to the size of the colour block. Higher values refer to a higher level of agreement and are represented by the darker blue colour blocks. Values equal to or below zero, represented by the white colour block and

burnt orange colour blocks, respectively, demonstrate a level of agreement worse than expected by chance.

In order to determine if the disagreement was attributable to a particular subject or group of subjects, the number of disagreements in each patient was plotted across all cohorts. The results demonstrate a higher observation disagreement (ML/ML/60, ML/ML/33, ML/AV/33, AV/AV10) in the subjects clinically classified as epileptic (Figure 3.17A-D). Paradoxically, there was one exception. The only patient in our study with a particularly outstanding disagreement (ML/ML/60, ML/ML/33 and ML/AV/33) belongs to the non-epilepsy cohort (20131125_1051, Figure 3.17A-C).

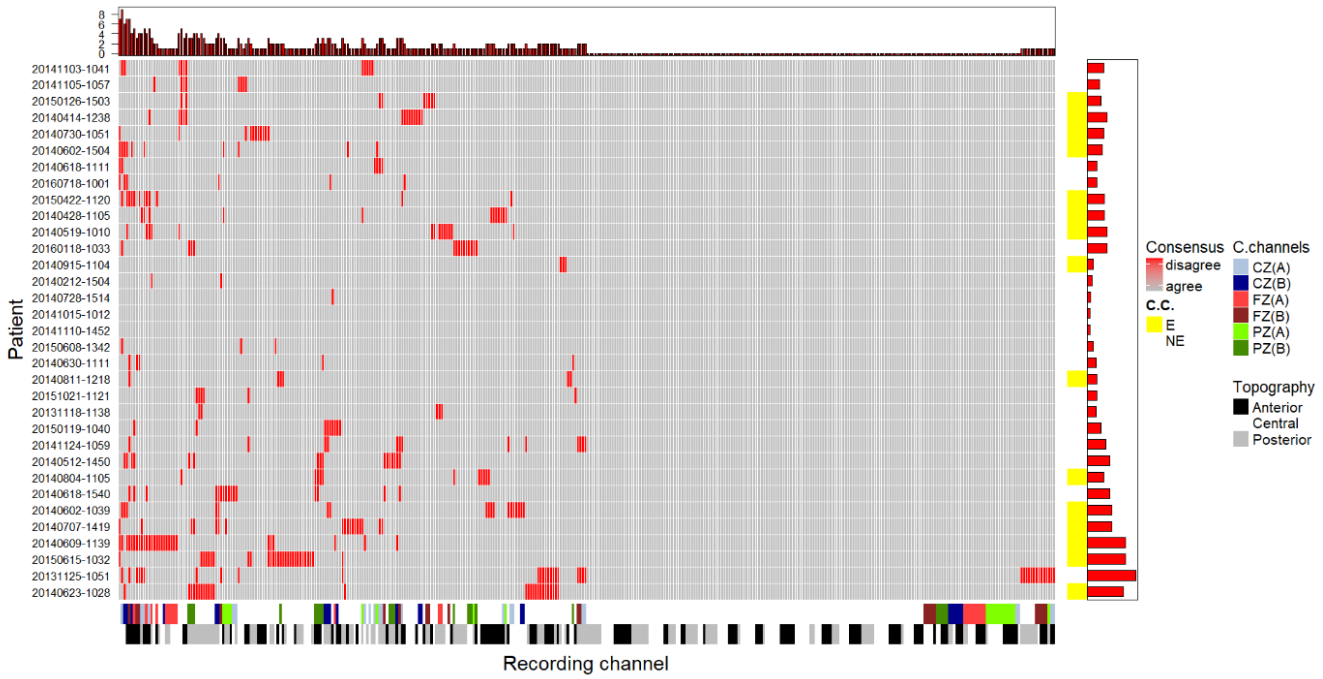
A.)

Disagreement heatmap (ML/ML/60)



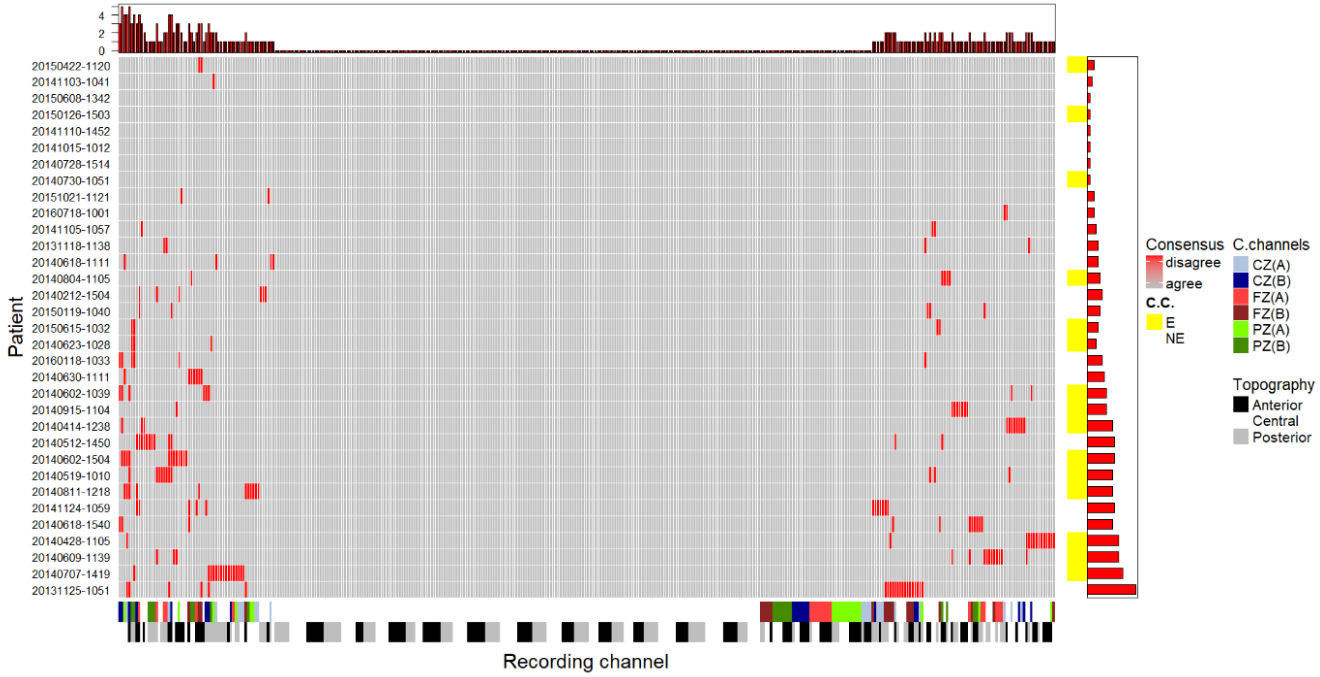
B.)

Disagreement heatmap (ML/ML/33)



C.)

Disagreement heatmap (ML/AV/33)



D.)

Disagreement heatmap (AV/AV/10)

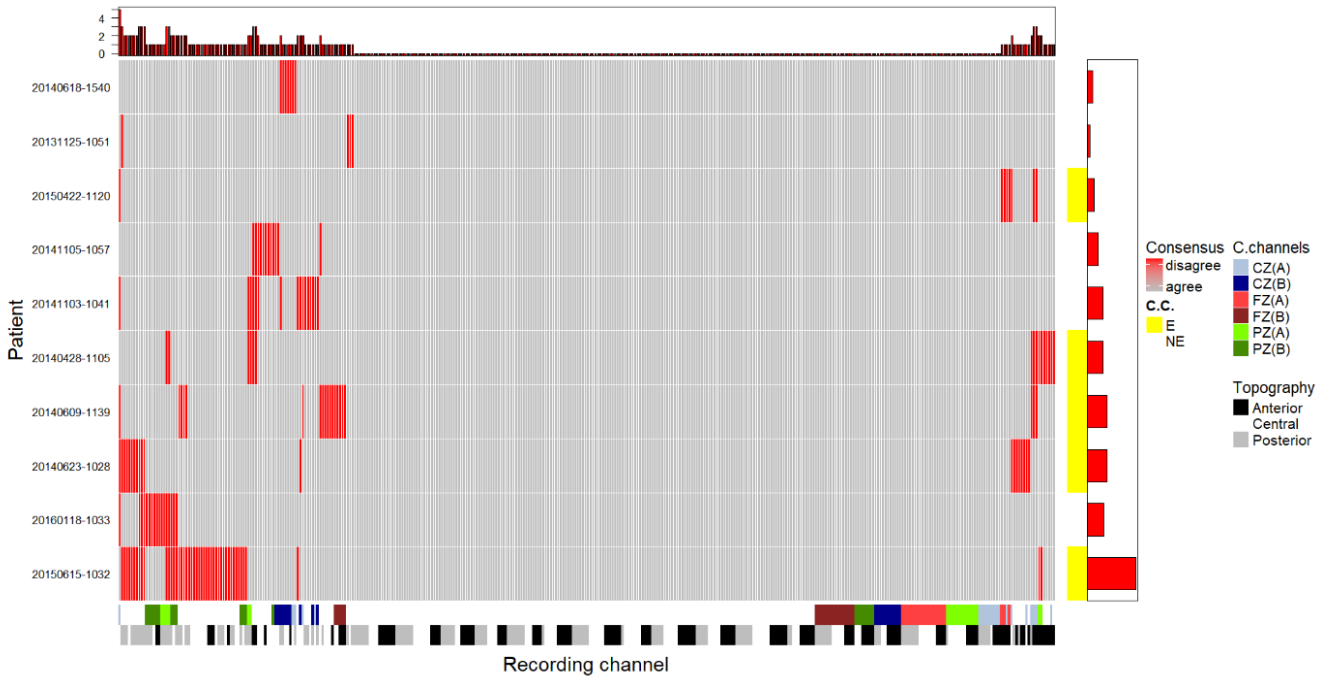


Figure 3.17. Disagreement heatmap representing the data points for individual subjects. A. Intra-observer disagreement in the full sample of 60 subjects for observer ML (ML/ML/60). **B.** Intra-observer disagreement in a sample of 33 subjects with complete data set for observer ML (ML/ML/33). **C.** Inter-observer disagreement between two independent observers on the sample of 33 subjects with complete data (ML/AV/33). **D.** Intra-observer disagreement on ten patients for observer AV (AV/AV/10). The abbreviations are as follows, clinical classification (C.C.), Epileptic (E), No epileptic (NE), central channels (C. Channels). C. Channels refers to the stimulation areas. Topography refers to the recording channels. In each heatmap figure, the Y-axis displays the subjects, and the X-axis represents the 378 data points. The colour in each square corresponds to the level of disagreement in each data point represented by the red colour blocks. The graph on the right margin of the heatmap represents the amount of data point disagreement in each particular subject compared to the rest of the subjects in the sample. The graph on the top margin of the heatmap represents the amount of disagreement in each particular data point.

To assess if the intra-observer and inter-observer variability for the detection of new-onset repetitive rhythms (NORRs) at each of the 378 recording points has a significant impact on the inter-observer variability for the final classification of RRs based on the definition of RRs (three or more recording channels displaying new-onset repetitive rhythms following TMS stimulation), the inter-observer (ML/AV/60) variability was also calculated for the presence of RRs at each stimulation channel (S.C.), (Figure 3.15C). The inter-observer (ML/AV/60) variability for the final TMS-EEG classification of positive or abnormal TMS-EEG study based on RRs alone (defined as RRs present after stimulation of at least one of the stimulation channels) was also calculated, and the level of agreement was weak (kappa=0.560), (Figure 3.15D).

	CZ (A)	PZ (A)	FZ (A)	CZ (B)	PZ (B)	FZ (B)	C3	C4	P3	P4	O1	O2	T5	T6	F3	F4	T3	T4	Predicted status (ISRR)	C.C.	Routine EEG
20140414_1238	4	0	0	3	0	0	0	0	0	1	0	0	0	0	5	0	0	0	E	E	Y
20140428_1105	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	E	E	Y
20140519_1010	0	0	3	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0	E	E	Y
20140707_1419	1	0	0	0	0	0	5	0	0	2	0	0	0	0	0	0	0	0	E	E	Y
20140804_1105	0	0	0	0	2	0	0	0	0	0	0	0	0	0	3	0	2	0	E	E	Y
20141124_1518	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	E	E	Y
20150121_1024	0	0	4	1	0	0	0	0	0	0	0	0	1	0	0	0	2	0	E	E	Y
20150422_1120	0	0	3	2	0	1	0	0	0	0	0	0	0	0	0	0	0	0	E	E	Y
20151214_1500	4	0	3	4	3	3	0	0	0	0	0	0	0	0	0	0	0	0	E	E	Y
20160125_1355	0	1	0	0	0	0	5	0	0	0	0	0	0	0	0	0	0	1	E	E	Y
20140602_1039	2	0	0	7	0	0	0	1	1	0	0	0	0	0	0	4	0	0	E	E	Y
20140623_1028	0	0	0	3	0	0	0	3	0	0	0	0	0	0	0	0	0	0	E	E	Y
20150126_1503	0	0	4	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	E	E	Y
20140609_1139	0	0	4	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	E	E	N
20141201_1434	0	0	0	0	3	4	0	0	0	0	0	0	0	2	0	0	0	0	E	E	N
20150615_1032	0	0	0	0	1	0	0	0	3	0	2	0	0	0	0	0	0	0	E	E	N
20140730_1051	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	E	E	N
20160413_1524	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	2	0	0	NE	E	Y
20140714_1444	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	NE	E	Y
20141201_1046	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	NE	E	Y
20140331_1113	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	NE	E	N
20140407_1114	0	0	0	2	0	0	0	0	0	2	1	0	0	0	0	0	0	0	NE	E	N
20140602_1504	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	NE	E	N
20140630_1438	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	NE	E	N
20140811_1218	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	NE	E	N
20140915_1104	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	NE	E	N
20140514_1035	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	NE	E	N
20140512_1450	0	0	0	2	4	0	0	0	0	0	0	0	0	0	0	0	0	0	E	NE	Y
20140908_1459	2	0	0	2	0	0	0	0	3	0	0	0	0	0	0	0	0	0	E	NE	Y
20140618_1540	0	3	2	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	E	NE	N
20150119_1040	0	0	0	3	0	0	0	0	2	0	2	0	0	0	0	0	0	0	E	NE	N
20160118_1033	1	7	0	0	10	0	0	0	0	0	0	5	3	0	0	0	0	0	E	NE	N
20131125_1051	0	0	0	0	0	2	2	2	0	0	0	0	0	0	0	0	0	0	NE	NE	N
20140507_1107	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	NE	NE	N
20140829_1555	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	NE	NE	N
20140910_1038	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	NE	NE	N
20151021_1121	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	NE	NE	N
20131118_1138	2	0	0	0	0	0	0	0	0	0	2	0	0	0	0	2	0	0	NE	NE	N
20140120_1112	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	NE	NE	N
20140212_1504	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	NE	NE	N
20140430_1108	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	NE	NE	N
20140512_1128	2	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	NE	NE	N
20140618_1111	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	NE	NE	N
20140630_1111	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	NE	NE	N
20140728_1514	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	NE	NE	N
20140804_1601	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	NE	NE	N
20140818_1010	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	NE	NE	N
20141015_1012	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	NE	NE	N
20141029_1040	2	0	1	1	0	2	0	0	0	0	0	0	0	0	0	0	0	0	NE	NE	N
20141103_1041	0	2	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	NE	NE	N
20141105_1057	0	0	0	0	1	0	0	0	0	0	1	0	0	0	2	0	0	1	NE	NE	N
20141110_1452	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	NE	NE	N
20141124_1059	2	0	0	2	0	0	0	0	0	0	0	0	2	0	0	0	0	0	NE	NE	N
20141126_1025	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	NE	NE	N
20150309_1514	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	NE	NE	N
20150518_1059	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	NE	NE	N
20150608_1342	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	NE	NE	N
20150710_1355	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	NE	NE	N
20151019_1047	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	NE	NE	N
20160718_1001	2	2	0	0	0	0	1	0	0	2	0	0	0	0	0	0	0	0	NE	NE	N

Table 3.3. Summary table of the 60 subjects in which the visual analysis was performed. The columns represent the stimulation channels, and the rows represent the subjects in the cohort study. The subjects displaying at least three new-onset repetitive rhythmic responses in a given stimulation channel (red colour block) were given a predictive TMS supported status

of likely epileptic (ISRR) represented in green colour. This predicted status was evaluated against the final clinical diagnosis (C.C.) and the baseline EEG results (Y=presence of epileptiform activity, represented in green colour).

		CZ (A)	PZ (A)	FZ (A)	CZ (B)	PZ (B)	FZ (B)	C3	C4	P3	P4	O1	O2	T5	T6	F3	F4	T3	T4	NTD	AED	DRs	RRs	TMS-EEG-NSDs	TMS-EEG-IEDs	Predicted status (ISRR)	C.C.	C.C.	Routine-EEG-NSDs	Routine-EEG-IEDs	Routine EEG
20140414_1238	7	4	0	0	3	0	0	0	0	0	1	0	0	0	0	5	0	0	0		LVT	Y	Y		Y	E	E	GGE/JAE	Y	Y	Y
20140428_1105	8	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		LVT	Y	Y		Y	E	E	GGE/JME		Y	Y
20140519_1010	14	0	0	3	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0		PHB, LVT	N	Y		Y	E	E	GGE/JAE		Y	Y
20140707_1419	23	1	0	0	0	0	0	5	0	0	2	0	0	0	0	0	0	0	0		VP		Y		Y	E	E	F/R TLE		Y	Y
20140804_1105	27	0	0	0	0	2	0	0	0	0	0	0	0	0	0	3	0	2	0				Y		Y	E	E	GGE/PA		Y	Y
20141124_1518	43	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0				Y		Y	E	E	F/ R FLE+L TLE (BF)		Y	Y
20150121_1024	48	0	0	4	1	0	0	0	0	0	0	0	0	1	0	0	0	2	0				Y		Y	E	E	F/R TLE		Y	Y
20150422_1120	51	0	0	3	2	0	1	0	0	0	0	0	0	0	0	0	0	0	0			N	Y		Y	E	E	GGE/GTCS only		Y	Y
20151214_1500	58	4	0	3	4	3	3	0	0	0	0	0	0	0	0	0	0	0	0				Y		Y	E	E	GGE/GTCS only		Y	Y
20160125_1355	60	0	1	0	0	0	0	5	0	0	0	0	0	0	0	0	0	0	1		LVT		Y		Y	E	E	GGE/GTCS only		Y	Y
20140602_1039	15	2	0	0	7	0	0	0	1	1	0	0	0	0	0	0	4	0	0		VP,LVT, LTG	Y	Y		Y	E	E	GGE/ELMA		Y	Y
20140623_1028	20	0	0	0	3	0	0	0	3	0	0	0	0	0	0	0	0	0	0				Y		Y	E	E	F/FLE		Y	Y
20150126_1503	49	0	0	4	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0		VP		Y		Y	E	E	F/ L H		Y	Y
20140609_1139	17	0	0	4	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0		VP		Y			E	E	IGE/GTCS only			N
20141201_1434	46	0	0	0	0	3	4	0	0	0	0	0	0	0	2	0	0	0	0		CBZ		Y	Y		E	E	F/R FT	NSD		N
20150615_1032	54	0	0	0	0	1	0	0	0	3	0	2	0	0	0	0	0	0	0				Y			E	E	GE (NC)			N
20140730_1051	26	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0		CBZ	Y	Y	Y		E	E	GGE/GTCS only	NSD		N
20160413_1524	61	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	2	0	0		LVT				Y	NE	E	F/ R FLE		Y	Y
20140714_1444	24	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		VP				Y	NE	E	GGE/ELMA		Y	Y
20141201_1046	45	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		LVY	N			Y	NE	E	GGE/JME		Y	Y
20140331_1113	5	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0		CBZ, LVT					NE	E	GGE/GTCS only			N
20140407_1114	6	0	0	0	2	0	0	0	0	0	2	1	0	0	0	0	0	0	0		LTG					NE	E	GE			N
20140602_1504	16	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0		CBZ, CLB			Y		NE	E	F/R TLE	NSD		N
20140630_1438	22	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0							NE	E	F/L FLE			N
20140811_1218	29	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0					Y (1)		NE	E	F/TLE			N
20140915_1104	34	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0							NE	E	F/L FLE			N
20140514_1035	13	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0							NE	E	PS mild	NSD		N
20140512_1450	12	0	0	0	2	4	0	0	0	0	0	0	0	0	0	0	0	0	0				Y	Y		E	NE	PNES	NSD L-MT		Y
20140908_1459	32	2	0	0	2	0	0	0	0	3	0	0	0	0	0	0	0	0	0		CBZ		Y	Y		E	NE	VVS	NSD		Y
20140618_1540	19	0	3	2	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0				Y			E	NE	VVS			N
20150119_1040	47	0	0	0	3	0	0	0	0	2	0	2	0	0	0	0	0	0	0				Y			E	NE				N
20160118_1033	59	1	7	0	0	10	0	0	0	0	0	0	5	3	0	0	0	0	0				Y	Y		E	NE	VVS	NSD		N
20131125_1051	2	0	0	0	0	0	2	2	2	0	0	0	0	0	0	0	0	0	0							NE	NE	PNES			N
20140507_1107	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0							NE	NE	AS			N
20140829_1555	31	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0							NE	NE	PNES			N
20140910_1038	33	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0							NE	NE	VVS			N
20151021_1121	57	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0		PGB					NE	NE	VVS			N
20131118_1138	1	2	0	0	0	0	0	0	0	0	0	2	0	0	0	0	2	0	0	5HT1						NE	NE	PNES			N
20140120_1112	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0							NE	NE	PNES			N
20140212_1504	4	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0							NE	NE	PNES			N
20140430_1108	9	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0							NE	NE	PNES			N
20140512_1128	11	2	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0							NE	NE	VVS			N
20140618_1111	18	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0							NE	NE	VVS			N
20140630_1111	21	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		VP					NE	NE	PNES			N
20140728_1514	25	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		CBZ			Y		NE	NE	AS	NSD		N
20140804_1601	28	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0							NE	NE	VVS/PNES			N
20140818_1010	30	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0					Y		NE	NE	VVS	NSD		N
20141015_1012	36	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0							NE	NE	VVS			N
20141029_1040	38	2	0	1	1	0	2	0	0	0	0	0	0	0	0	0	0	0	0							NE	NE	VVS			N
20141103_1041	39	0	2	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0							NE	NE	PNES			N
20141105_1057	40	0	0	0	0	1	0	0	0	0	0	1	0	0	0	2	0	0	1							NE	NE	VVS			N
20141110_1452	41	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0					Y		NE	NE	VVS	NSD		N
20141124_1059	42	2	0	0	2	0	0	0	0	0	0	0	0	2	0	0	0	0	0							NE	NE	PNES			N
20141126_1025	44	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0							NE	NE				N
20150309_1514	50	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0							NE	NE	AS			N
20150518_1059	52	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0							NE	NE	VVS			N
20150608_1342	53	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0							NE	NE	VVS			N
20150710_1355	55	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	SSRI						NE	NE	PNES			N
20151019_1047	56	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0							NE	NE	VVS			N
20160718_1001	62	2	2	0	0	0	0	1	0	0	2	0	0	0	0	0	0	0	0							NE	NE				N

Table 3.4. Summary table of the 60 subjects in which the visual analysis was performed. The columns represent the stimulation channels, and the rows represent the subjects in the cohort study. The subjects displaying repetitive responses in at least one of the stimulation channels (red colour block) were given a predictive TMS supported status of likely epileptic (ISRR) represented in green colour. This predicted status was evaluated against the final clinical diagnosis (C.C.) and the baseline EEG results (Y=abnormal, green colour; N=normal, white colour); E, epilepsy; NE, no epilepsy; IEDs, interictal epileptiform discharges (Y=present, green colour; no present, white colour); NSDs, nonspecific discharges; DRs, delayed responses; RRs, repetitive responses; R, right; L, left; F, focal epilepsy; GGE, genetic generalised epilepsy; JME, juvenile myoclonic epilepsy; JAE, juvenile absence epilepsy; ELMA, eyelid myoclonia and absence; GTCS, generalised tonic-clonic seizures; PA, phantom absences; NC, no specified subsyndrome; TLE, temporal lobe epilepsy; FLE, frontal lobe epilepsy; BF, bilateral focus; H, lateralising but non-localising; AS, acute symptomatic; PNES, psychogenic non-epileptic seizures; VVS, vasovagal syncope; PS, photosensitive; AED, antiepileptic drug; LTG, lamotrigine; LVT, levetiracetam; PHB, phenobarbital; VP, valproate

acid; CBZ, carbamazepine; CLB, Clobazam; PGB, Pregabalin; NTD, other neurotropic drugs; SSRI, selective serotonin reuptake inhibitor (Sertraline); 5HT1-receptor agonist.

C.3.7. Quantitative interpretation of TMS evoked EEG responses

Introduction

The EEG tracks and records the brain activities in a continuous high temporal resolution fashion. The Transcranial Magnetic Stimulation (TMS) pulse, which is used to change the local magnetic field at the targeted area, is believed to alter the electric charge of the brain upon the onset of the stimulation. Initially, visual inspection after signal averaging was the applied method to identify these TMS induced signal fluctuations. The change of patterns could stretch from noticeable to imperceptible to the human eye due to intermixed spectral frequencies (δ, θ, α, and β bands) and amplitude oscillations, making them difficult to be identified. Our initial visual analysis is prone to human error and is subjective to examiners' criteria, as proven by the previous analysis of the RRs.

Additional information of interest in the EEG data is usually hidden in the frequency and power domain. Hereby, a continuous Morlet wavelet transformation was carried out on the EEG data as described in the chapter materials and methods, quantitative section. By comparing the power after and before TMS stimulation as power ratio over the predetermined frequency spectrum, the effect of the TMS pulse on the brain wave can be evaluated.

C.3.7.1. Study cohort

In eight out of twenty-seven epileptic subjects and nine out of thirty-three non-epileptic subjects, the TMS study recoding protocol was incomplete due to the subjects' intolerance to the test or technical recording pitfalls. Therefore, a total of forty-three subjects had a complete data set. In this sub-cohort, no significant difference was observed in age and gender between epilepsy and non-epilepsy groups (Table 3.5).

Of the forty-three subjects with completed TMS-EEG, nineteen were diagnosed with epilepsy. Ten out of these nineteen epileptic patients had genetic generalised epilepsy (GGE). The ten GGE patients were further subdivided into one had juvenile myoclonic epilepsy (JME), two with juvenile absence epilepsy (JAE), one with epilepsy with eyelid myoclonia and absence (ELMA), three with genetic generalised epilepsy with generalised tonic-clonic seizures only and one with genetic generalised epilepsy with phantom absences (GGE-PA). In two patients, no GGE subsyndrome could be specified. The remaining nine epileptic patients were diagnosed with focal epilepsy (FE), of which four had temporal lobe epilepsy (TLE), four frontal lobe epilepsy (FLE) and one case had lateralising but non-localising focal epilepsy.

Of the twenty-four non-epileptic subjects with completed TMS-EEG recorded, ten were classified as psychogenic non-epileptic seizures (PNES), nine as vasovagal syncope (VVS), two as acute symptomatic seizures (AS) and the remaining three as non-epileptic but the final diagnoses remained uncertain (Table 3.5, Figure 3.18).

Upon visual inspection of the 43 completed TMS studies, eight studies were considered excessively noisy for the purpose of the quantitative analysis due to the excessive noise in some of the recording channels, and these were also rejected for the quantitative study. Therefore, 35 subjects (16 epilepsy and 19 non-epilepsy) were selected for the quantitative analysis.

	Epilepsy (n=19)	Non-Epilepsy (n=24)
Age	17 - 51 (29)	16 - 69 (29)
Gender (M: F)	9 : 10	11 : 13
Medication (Y: N)	10 : 9	5 : 19
Motor threshold	40 - 85 (53)	40 - 83 (61)
MRI (N: A: No)	13 : 5 : 1	20 : 1 : 3
Baseline EEG epileptic activity (Y: N)	11 : 8	1 : 23

Table 3.5. The table illustrates the following characteristics in the sample of 43 patients with a complete TMS-EEG study. Age, age range; Gender, gender ratio; Medication, use of antiepileptic medication at the time of the TMS study; Motor threshold, TMS resting motor threshold; MRI, MRI data (N, normal; A, abnormal; No, no performed); Baseline EEG epileptic activity, presence of epileptiform dischargers in the baseline EEG performed prior to the TMS-EEG study (Y, yes; N, no).

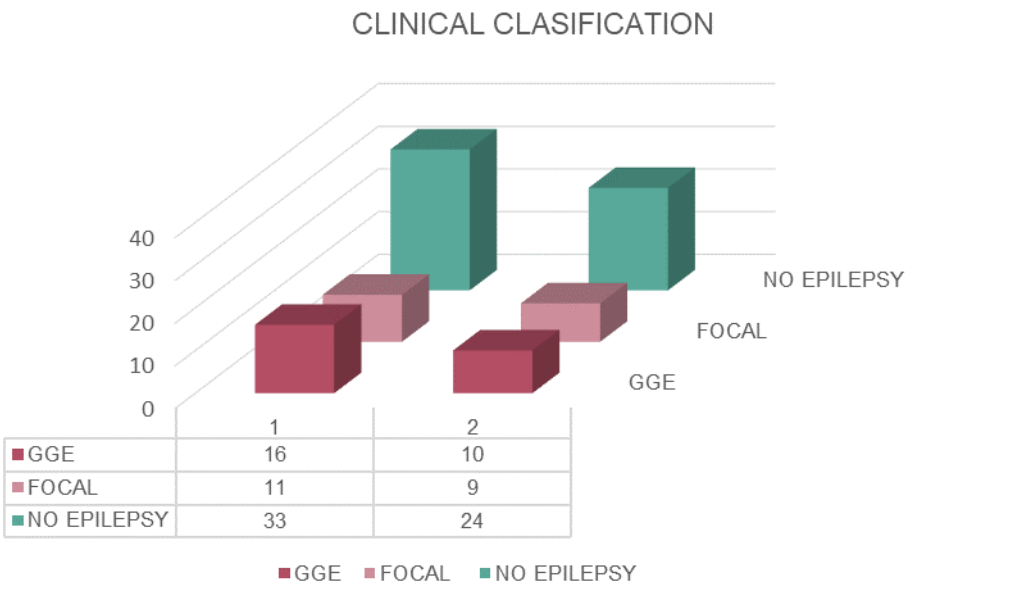


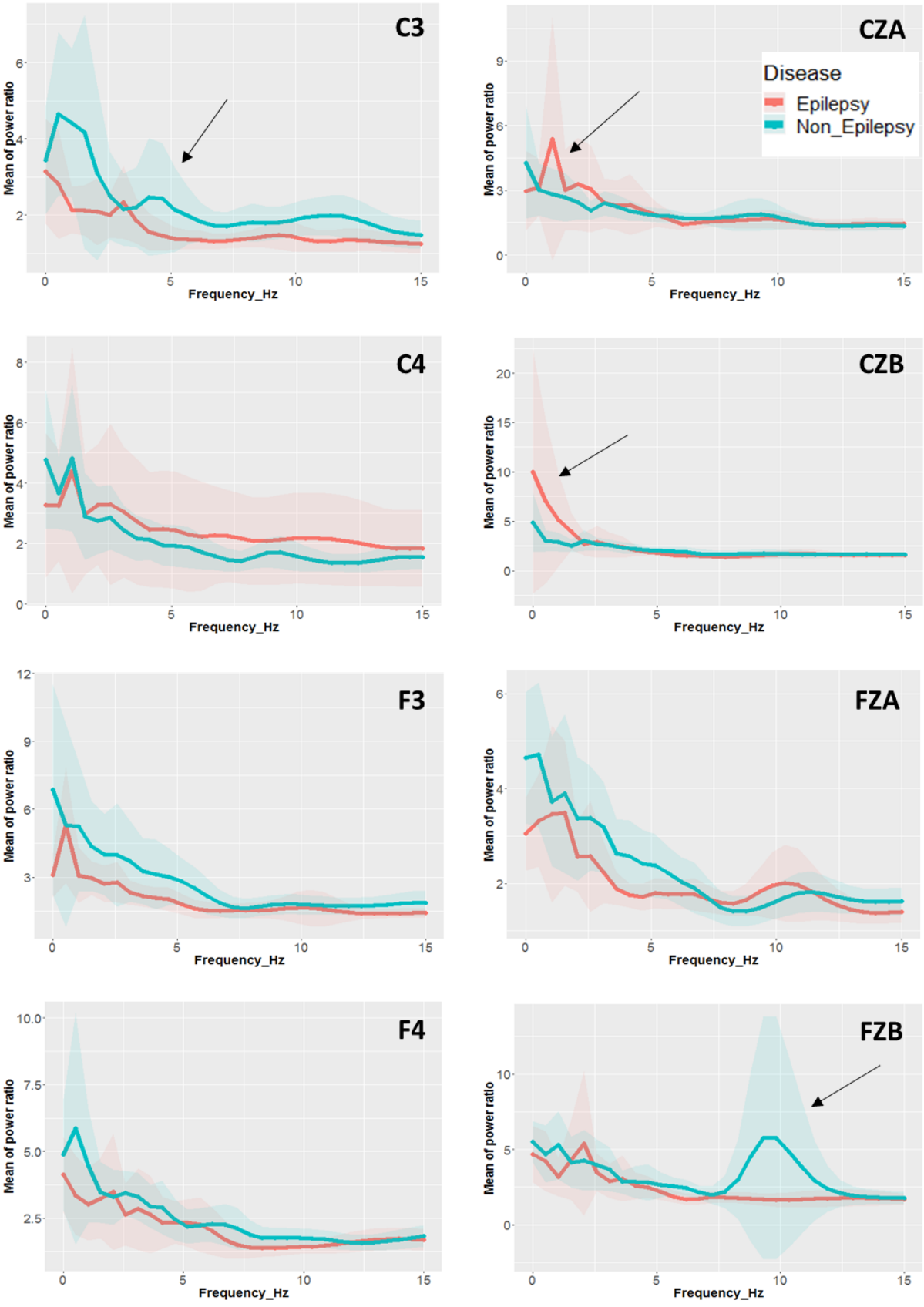
Figure 3.18. Clinical classification. The graph shows a similar clinical classification profile distribution between the full sample with 60 subjects used for the initial visual analyses of the RRs and the second cohort of 43 subjects with a complete TMS-EEG study, which will be the source for the subsequent quantitative analysis.

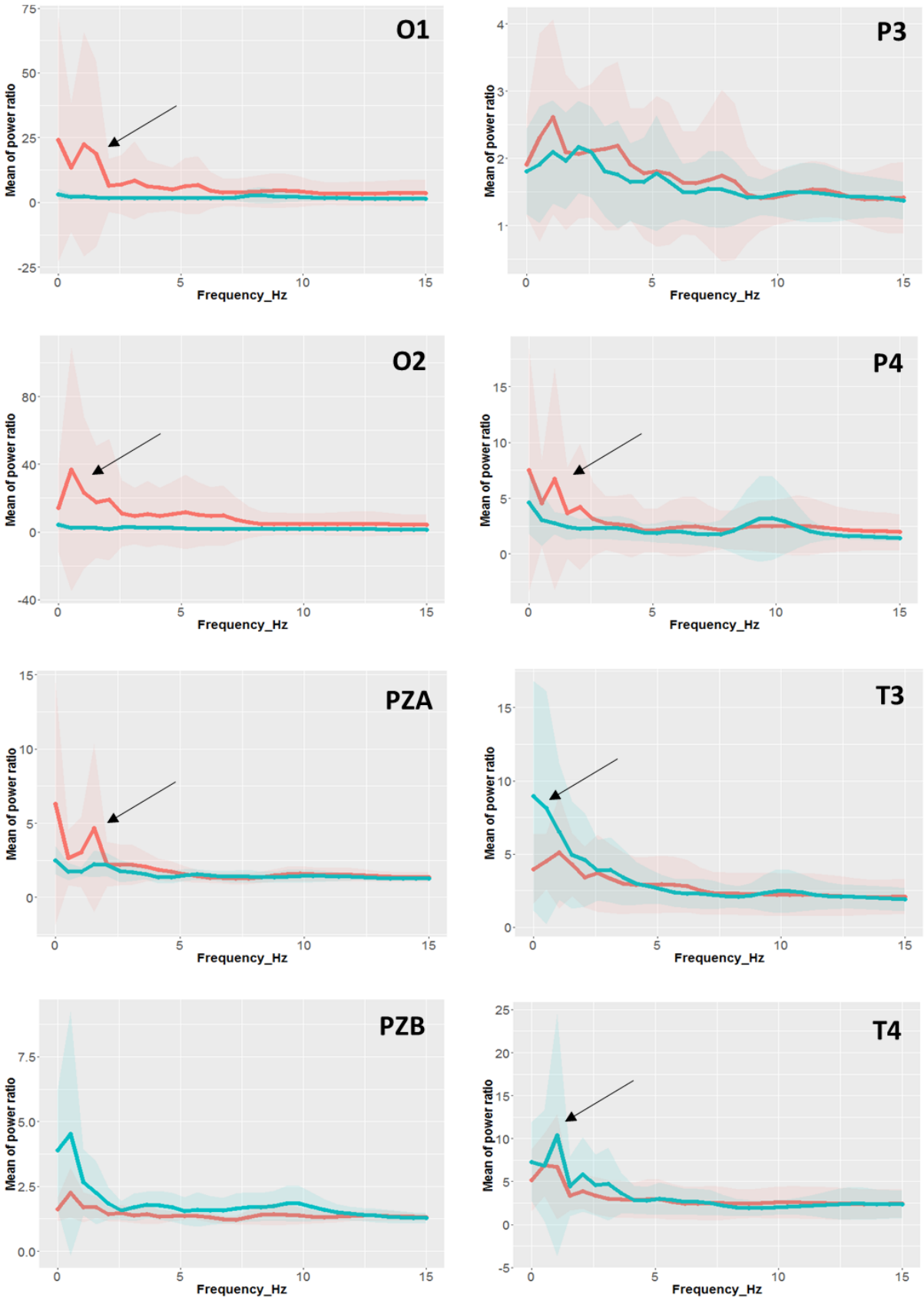
C.3.7.2. Grand average power-ratio in epilepsy and non-epilepsy subjects.

In order to appreciate the effect of TMS on EEG recording in epilepsy subjects, a grand average power ratio was calculated within each cohort by averaging the power ratio of all recording channels. The result was then plotted across the frequency spectrum (1 ~ 42 Hz) for each stimulation channel. Peaks of the curves along frequencies in either cohort were considered to be the epilepsy traits attributed to the TMS effect, and curves with monotonic behaviours were suggestive of no noticeable TMS impact. Likewise, the 95 % confidence interval was computed to determine whether the difference between the two means (epilepsy and non-epilepsy) was statistically significant. If those intervals overlapped, it was concluded that the difference between groups was not statistically significant and vice versa.

Upon visualisation (Figure 3.19 and Table 3.6), a trend of differences between epilepsy and non-epilepsy curves was observed at certain stimulation channels (SCs) and frequency bands. A peak in the power ratio curve was seen in the delta frequency band for the epilepsy group in the midline central (CzA, CzB), midline posterior (PzA) and posterior right parasagittal (P4) stimulation channels; and in the delta and theta waves in the posterior (O1 and O2) channels. Interestingly, this phenomenon was particularly noticeable in the posterior channels (O1 and O2) of the epilepsy cohort, in which the peak of the curve rose at low-frequency bands and declined along the higher frequency spectrum in comparison to the relatively flat line of the non-epilepsy group. In contrast to the epilepsy group, the non-epilepsy cohort demonstrated an upsurge of power ratio at a higher frequency range, alpha, with stimulation of the anterior midline (FzB) channel, as well as in the delta band over the central left parasagittal (C3) and anterior temporal (T3, T4) stimulation channels. In summary, the epilepsy group showed a higher power ratio in the lower frequency spectra (delta and theta), which is

particularly noticeable upon stimulation over the posterior (O1, O2, PzA) and midline central (CzB, CzA) regions. The no-epilepsy group showed a higher power ratio in the alpha frequency spectra upon stimulation over the anterior midline region (FzB). Both cohorts exhibited no difference in the power ratio in the T5 and T6 stimulation channels, suggesting that TMS stimulation on the posterior temporal channels might not be of paramount importance for epilepsy detection in this cohort.


C3
CZA
C4
CZB
F3
FZA
F4
FZB
Disease
Epilepsy
Non_Epilepsy
Mean of power ratio
Frequency_Hz


O1
P3
O2
P4
PZA
T3
PZB
T4
Mean of power ratio
Frequency_Hz

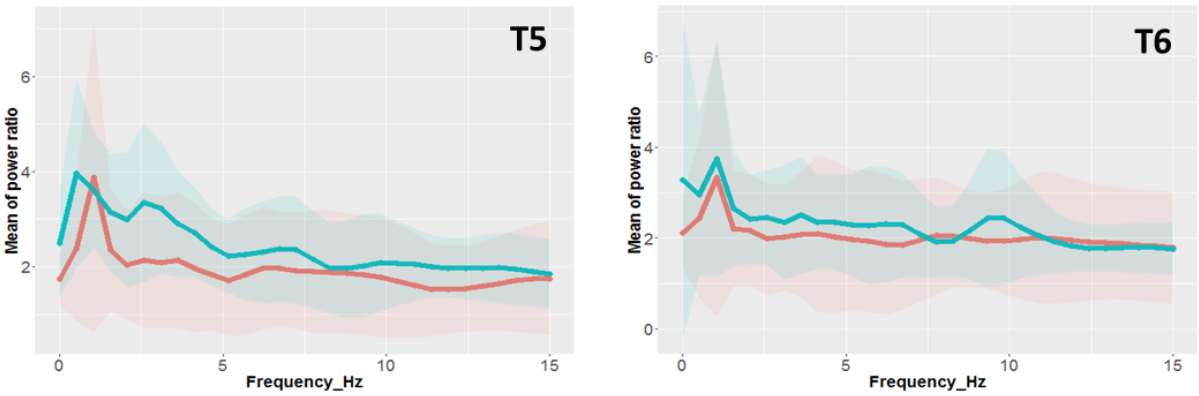


Figure 3.19. Grand average power-ratio over frequency spectrum in epilepsy and non-epilepsy subjects in the 18 TMS stimulation channels. In each individual figure, the X-axis represents the frequency ranges in Hz, and the Y-axis displays the values of the grand average power ratio. The coloured-filled line represents the means of epilepsy (red colour) and non-epilepsy (blue colour) cohorts across the frequency bins (spectrum), and the 95 % confidence interval (CI) is shown as the ribbon. The black arrows indicate the points where the mean between groups looks different with overlapping CI.

	C3	C4	FzA	FzB	CzA	CzB	PzA	PzB	F3	F4	O1	O2	P3	P4	T3	T4	T5	T6
E		=	=		↑ δ	↑ δ	↑ δ	=	=	=	↑ δ ↑ θ	↑ δ ↑ θ	=	↑ δ			=	=
NE	↑ θ ↑ δ	=	=	↑ α				=	=	=			=		↑ δ	↑ δ	=	=

Table 3.6. Summary of the grand average power-ratio findings in epilepsy (E) and non-epilepsy (NE) subjects in the 18 TMS stimulation channels. The colour denotes the topographic localisation of the TMS stimulation; parasagittal channels - orange, midline channels – yellow, temporal channels – green, posterior – white. Frequency band: α, Alpha (≥8, <14 Hz); θ, Theta (≥4, <8 Hz); δ, Delta (<4 Hz); ↑, increased grand average power ratio; ↓, decreased grand average power-ratio: =, no difference between the means; E, epilepsy group; NE, non-epilepsy group.

Overall, the grand average power ratio analysis revealed a differential trend between epilepsy and non-epilepsy cohorts in particular frequencies (delta, theta, alpha) and stimulation channels (C3, FzB, CzA, CzB, PzA, T3, T4, O1, O2 and P4). These findings failed to reach a level of significance due to the nature of the data. The phase cancellation phenomena derived from the average of all of the recording channels may obscure the results. Arithmetic mean is the simplest tool to measure central tendency in a data set. It works well only when all the values are approximately in a close range. However, it is not the best measurement to use in the datasets containing extreme values or dispersed data points in general. To circumvent this limitation, a new approach to assess the impact of the TMS stimulation at a particular channel in a given stimulus point and its association with epilepsy is needed.

C.3.7.3. Generation of a machine learning-based classification model for epilepsy prediction

Machine learning-based classification is the use of algorithms that learn how to assign a class of labels from the input values given for training. It will predict the class labels under which new data will fall. To perform the model generation and unravel if the TMS aids in the classification prediction, a power-ratio based TMS-EEG dataset was constructed, consisting of 35 subjects/rows and 1890 variables/columns (5 frequency bands x 21 recording channels x 18 stimulation points).

C.3.7.4. Preliminary variable selection

With the intention of improving the performance of the machine learning algorithm, a crude variable pre-selection step was implemented using the non-parametric Wilcoxon Rank Sum Test between epilepsy and non-epilepsy cohorts. 60 out of 1890 variables initially passed the benchmark and had a p-value smaller than 0.05. Regrettably, none

of these variables survived from multiple comparison correction (neither Bonferroni correction or less stringent Benjamini-Hochberg (False Discovery Rate) procedure, $p.adj < 0.05$).

This preliminary variable selection was an exploratory attempt at finding variables associated with the disease. In order to interrogate if these short-listed variables hold a biological meaning, the data was explored with unsupervised hierarchical clustering analysis together with the clinical diagnosis information. The result unambiguously showed that these variables were indeed associated with the disease and were capable of separating the study subjects into epilepsy and non-epilepsy groups. Additionally, two mega clusters of variables were also identified, which displayed an inverse relationship of power ratio between disease status (Figure 3.20). Knowing the potential of these preliminary variables for disease classification, a more sophisticated approach to the variable selection stage was essential to facilitate and boost the accuracy of the machine learning model for epilepsy prediction.

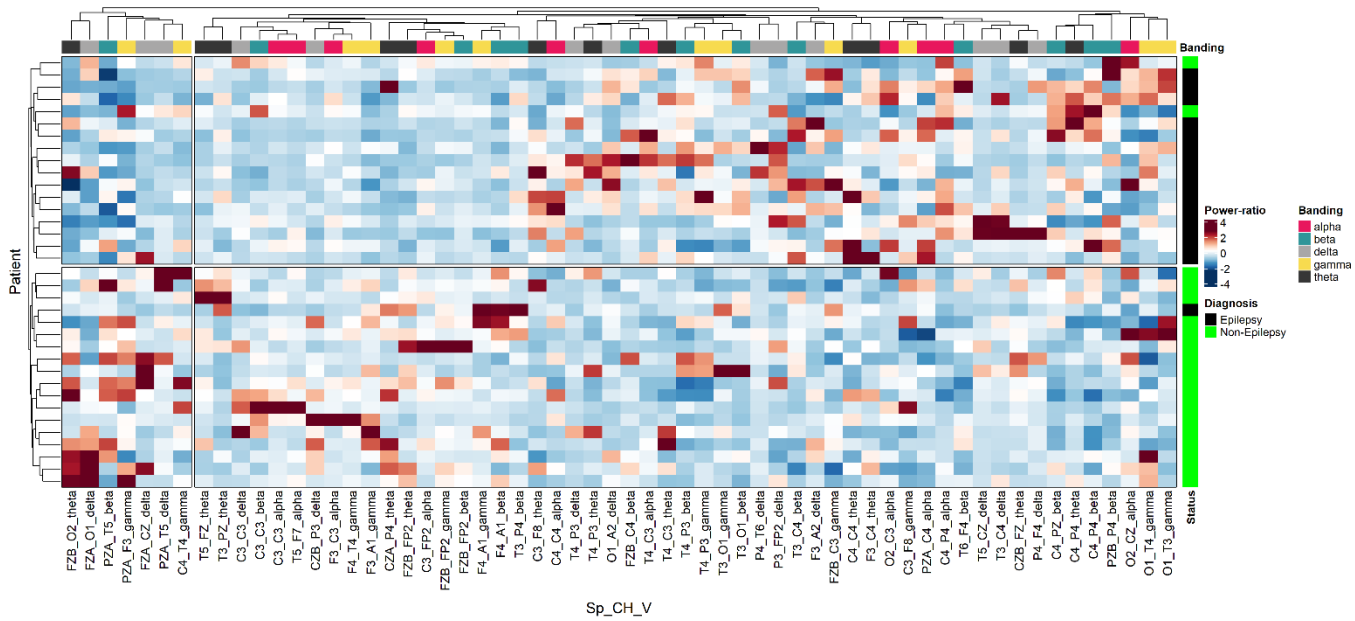


Figure 3.20. The unsupervised hierarchical clustering analysis shows the 60 selected variables were able to separate the 35 subjects' samples into two distinct cohorts. The X-axis represents the selected variables derived from the TMS stimulation at a specific stimulation point (Sp, total 18 stimulation targets), recorded in a particular channel (CH) at a particular frequency band (V, total 5 bins: delta, theta, alpha, beta, gamma). The Y-axis displays the subjects.

C.3.7.5. Selection of epilepsy-associated features for machine learning models

A model with too many variables is likely to overfit the idiosyncratic features of the training dataset and, therefore, may not perform well on new data. Additionally, the redundant features usually lead to noisy data resulting in model inaccuracy. Thus, it is desirable to have simple models that generalise well and, in turn, input data with fewer input variables. With this aim in mind, a new approach to variable selection was introduced. The strategy was to utilise two Cross-validation (CV) methods together with the Wilcoxon Rank Sum Test for model evaluation in feature selection (see material and methods chapter for details). Variables with a p-value smaller than 0.05 in at least ten training datasets were selected. In sum, 28 variables and 27 variables

met the criteria in fivefold Cross-validation (FFCV) and Monte Carlo Cross-validation (MCCV) methods, respectively. Only the 23 common variables identified by both FFCV and MCCV methods were further subjected to downstream analyses, including exploration of disease association and generation of the classification model for disease prediction (Figure 3.21A). The 23 were within the previously selected 60, suggesting that the new approach offers the advantage of selecting the variables more strongly associated with epilepsy.

The unsupervised hierarchical clustering performed on those 23 variables revealed two distinct clusters in the variables separating the subjects into two groups based on the disease status (Fisher exact test $p < 0.00001$) (Figure 3.22A).

In order to test if these 23 variables were important for disease recognition, an epilepsy prediction model was generated using the support vector machine (SVM) with linear kernel algorithm along with FFCV and MCCV re-sampling methods in the R language packages (CMA). The model generated in each training set was tested on the validation set in each iteration resulting in 700 observations in the FFCC and MCCV, (each training set had 28 subjects, 4/5 of 35 total number of subjects, and each validation test set had seven subjects, 1/5 of 35 toral number of subjects). The number of misclassifications was 100 over 700 observations in both FFCV and MCCV, rendering a misclassification rate of 0.143. Furthermore, 421 out of the 460 true positive observations in the FFCV were correctly classified as epileptic, resulting in a sensitivity of 0.897, while 259 out of the 320 true negative observations were correctly classified as non-epileptic, resulting in a specificity of 0.809. Similarly, 355 observations in the MCCV were correctly classified as epileptic over the 400 observations with the condition, giving a sensitivity of 0.887 and 245 observations

were correctly classified as non-epileptic over the 300 observations without the condition, resulting in a specificity of 0.817 (Figure 3.23A).

Apart from the accuracy, other measures such as the average probability of correct classification and the area under the receiver operating curve (ROC) were also estimated to measure the performance of the classifier. A value of 0.7 in the average probability and an AUC of 0.9 were computed in both re-sampling methods (Figure 3.23B-C), suggesting the superiority of the SVM classifier in distinguishing between epilepsy and non-epilepsy classes.

C.3.7.6. Sham-TMS Experiment

It could be argued that our classifier relies more strongly on the underlying idiosyncratic differences in frequency EEG rhythms within epileptic and non-epileptic subjects than on a true effect of the TMS stimulus paradigm. So as to test if the TMS protocol contributes to disease detection by enhancing the cortical hyperexcitability in the epileptic subjects, which otherwise would be undetectable by the classifier, a new power ratio dataset without the TMS effect referred to as Sham-TMS was generated.

When unsupervised hierarchical clustering analysis was performed over the Sham-TMS records, using the same 23 variables extracted from the TMS dataset, it failed to form meaningful clusters in both variables and subjects. Despite being partitioned into two categories, the subjects with epilepsy condition were dispersed between clusters (Fisher exact test, p=0.716), suggesting that TMS is vital in these epilepsy-associated variables (Figure 3.22B).

So as to investigate if the EEG recording alone could also predict epilepsy, the same analyses for feature selection and SVM classifier were carried out in the Sham dataset. On this occasion, 23 and 25 variables were selected in FFCV and MMCV, respectively

(Figure 3.21B). Twenty-two variables were common to both methods. These Sham-TMS selected variables were different from those selected with TMS (Figure 3.25) and also were able to separate the subjects into epilepsy and non-epilepsy groups with two different sets of variables, as seen in Figure 3.22C. The performance of the classifier in the Sham dataset was also assessed (Figure 3.23D-F), and the results suggested that the Sham SVM classifier worked well at distinguishing between epilepsy and non-epilepsy classes.

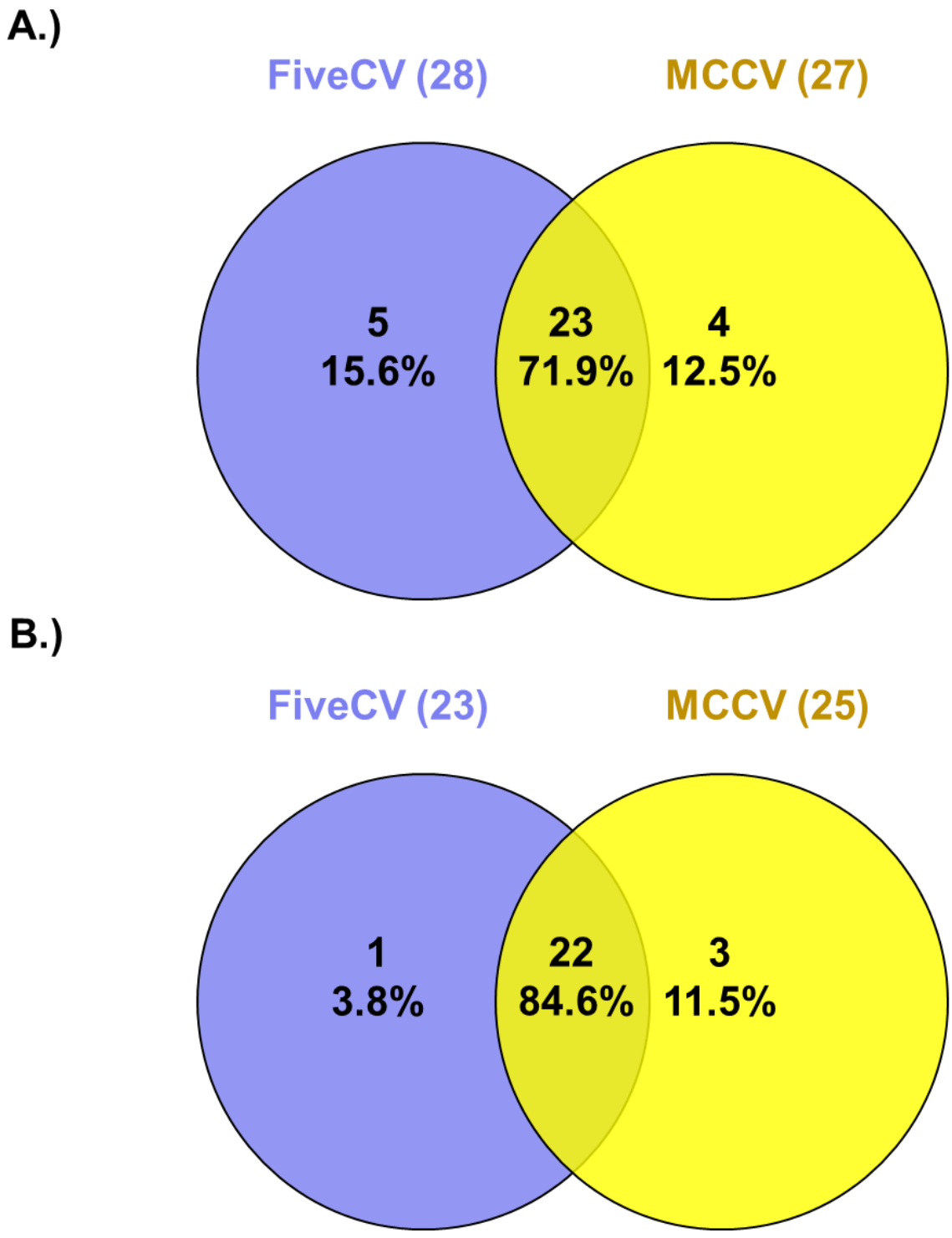
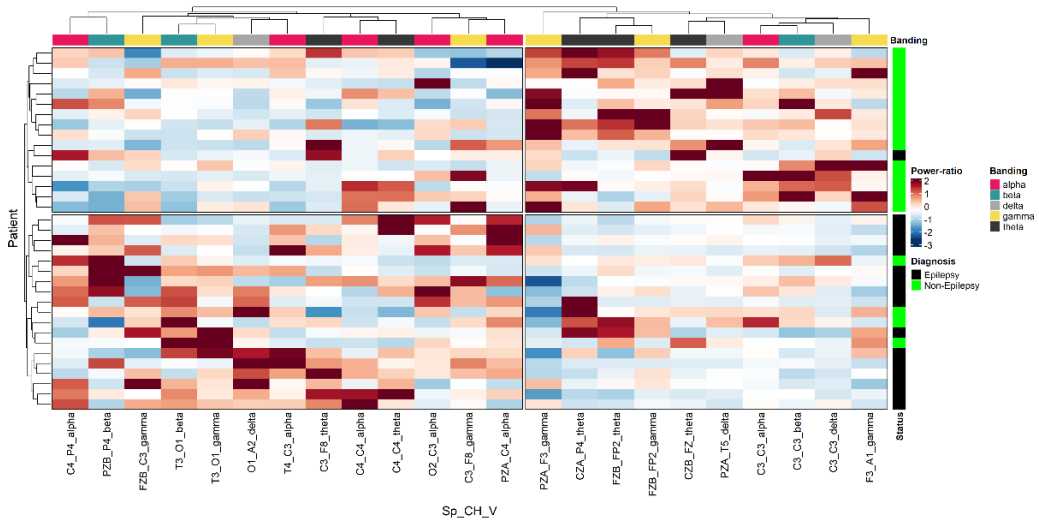
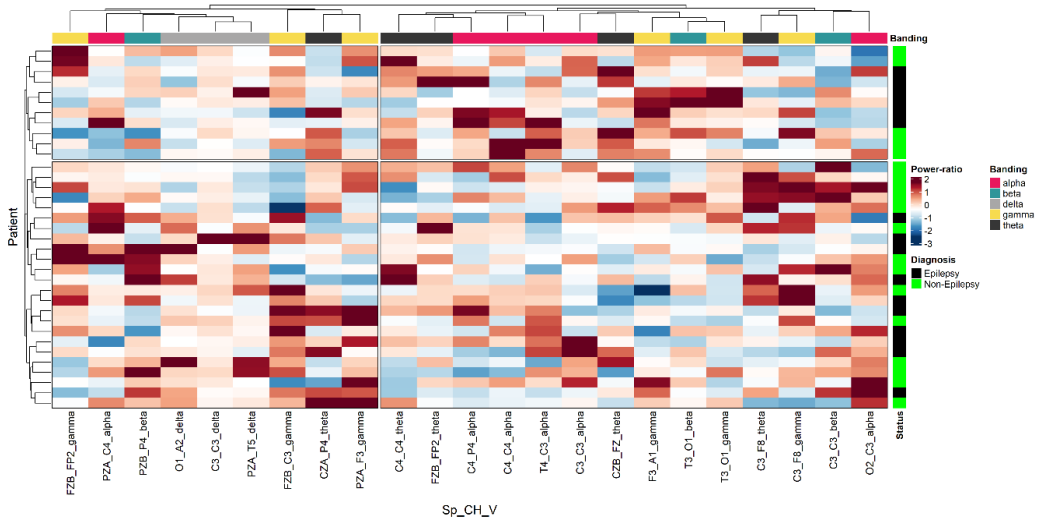


Figure 3.21. Feature selection for SVM classification models. The Venn diagrams represent the variables selected by FFCV (blue circle) and MCCV (yellow circle) methods in the TMS **(A)** and Sham-TMS experiments **(B)**.

A.)


Banding
Power-ratio
2
1
0
-1
-2
-3
Banding
alpha
beta
delta
gamma
theta
Diagnosis
Epilepsy
Non-Epilepsy
Patient
C4_P4_alpha
PZB_P4_beta
FZB_C3_gamma
T3_O1_beta
T3_O1_gamma
O1_A2_delta
T4_C3_alpha
C3_F8_theta
C4_C4_alpha
C4_C4_theta
O2_C3_alpha
C3_F8_gamma
PZA_C4_alpha
PZA_F3_gamma
CZA_P4_theta
FZB_FP2_theta
FZB_FP2_gamma
CZB_FZ_theta
PZA_T5_delta
C3_C3_alpha
C3_C3_beta
C3_C3_delta
F3_A1_gamma
Status
Sp_CH_V

B.)


Banding
Power-ratio
2
1
0
-1
-2
-3
Banding
alpha
beta
delta
gamma
theta
Diagnosis
Epilepsy
Non-Epilepsy
Patient
FZB_FP2_gamma
PZA_O4_alpha
PZB_P4_beta
O1_A2_delta
C3_C3_delta
PZA_T5_delta
FZB_C3_gamma
CZA_P4_theta
PZA_F3_gamma
C4_C4_theta
FZB_FP2_theta
C4_P4_alpha
C4_C4_alpha
T4_C3_alpha
C3_C3_alpha
CZB_FZ_theta
F3_A1_gamma
T3_O1_beta
T3_O1_gamma
C3_F8_theta
C3_F8_gamma
C3_C3_beta
O2_C3_alpha
Status
Sp_CH_V

C.)

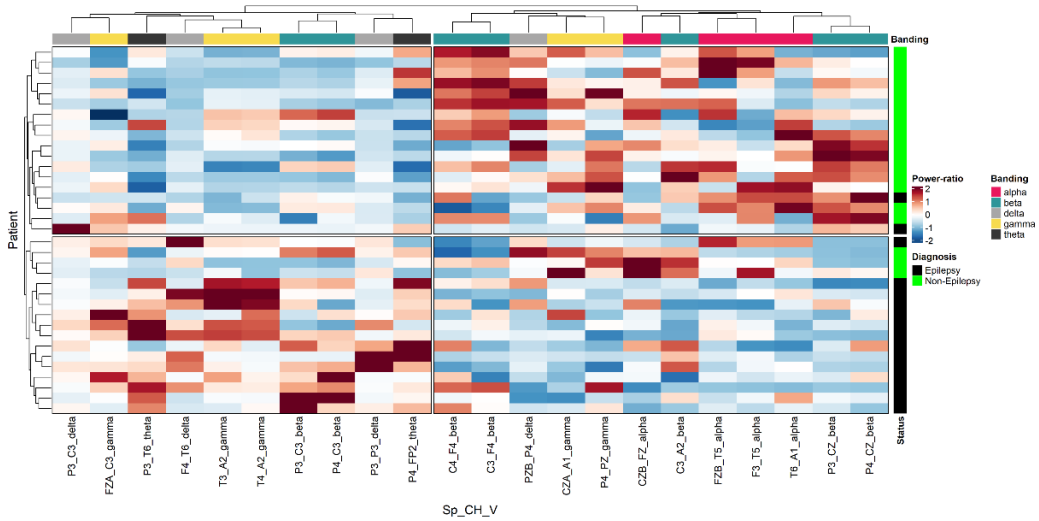
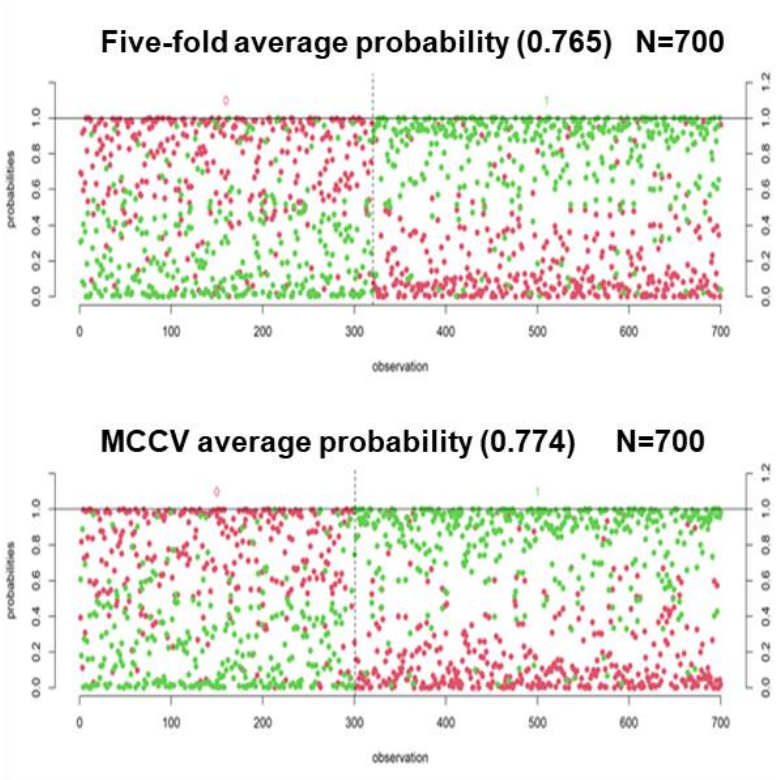

Banding
Power-ratio
2
1
0
-1
-2
Banding
alpha
beta
delta
gamma
theta
Diagnosis
Epilepsy
Non-Epilepsy
Patient
P3_C3_delta
FZA_C3_gamma
P3_T6_theta
F4_T6_delta
T3_A2_gamma
T4_A2_gamma
P3_C3_beta
P4_C3_beta
P3_P3_delta
P4_FP2_theta
C4_F4_beta
C3_F4_beta
PZB_P4_delta
CZA_A1_gamma
P4_PZ_gamma
CZB_FZ_alpha
C3_A2_beta
FZB_T5_alpha
F3_T5_alpha
T6_A1_alpha
P3_CZ_beta
P4_CZ_beta
Status
Sp_CH_V

Figure 3.22. Exploration of disease association features by unsupervised hierarchical clustering analysis. Unsupervised hierarchical clustering analysis to explore epilepsy-associated observation clusters in **A.)** the 23 variables identified in the TMS dataset, **B.)** the same 23 variables in the Sham-TMS dataset, and **C.)** the 22 variables selected in the Sham experiment during feature selection for the classification model. The X-axis are the variables arranged by the stimulation point (Sp, total 18 stimulation targets), recording channel (CH, total 21) and frequency band (V, total 5 bins: delta, theta, alpha, beta, gamma) separated by an underscore. The Y-axis displays the subjects, the non-epileptic labelled in green and the epileptic highlighted in black.

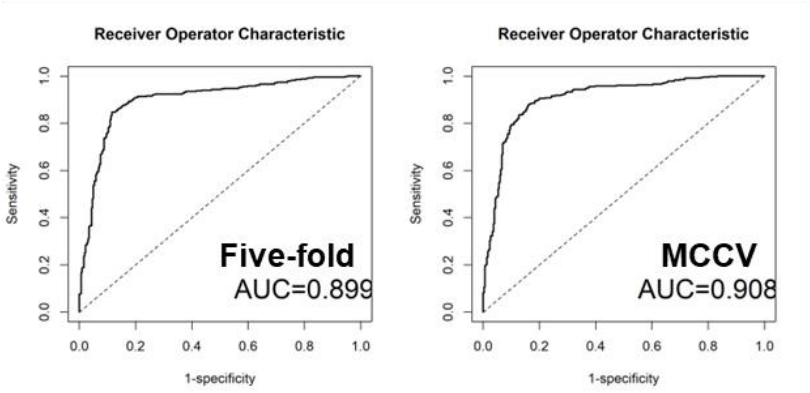
A.)

Five-fold CV				MCCV			
number of missclassifications: 100				number of missclassifications: 100			
missclassification rate: 0.143				missclassification rate: 0.143			
sensitivity: 0.897				sensitivity: 0.887			
specificity: 0.809				specificity: 0.817			
		predicted				predicted	
		0	1			0	1
true	0	259	61	true	0	245	55
	1	39	421		1	45	355

B.)



C.)



D.)

Five-fold CV

number of missclassifications: 6
missclassification rate: 0.009
sensitivity: 0.997
specificity: 0.984

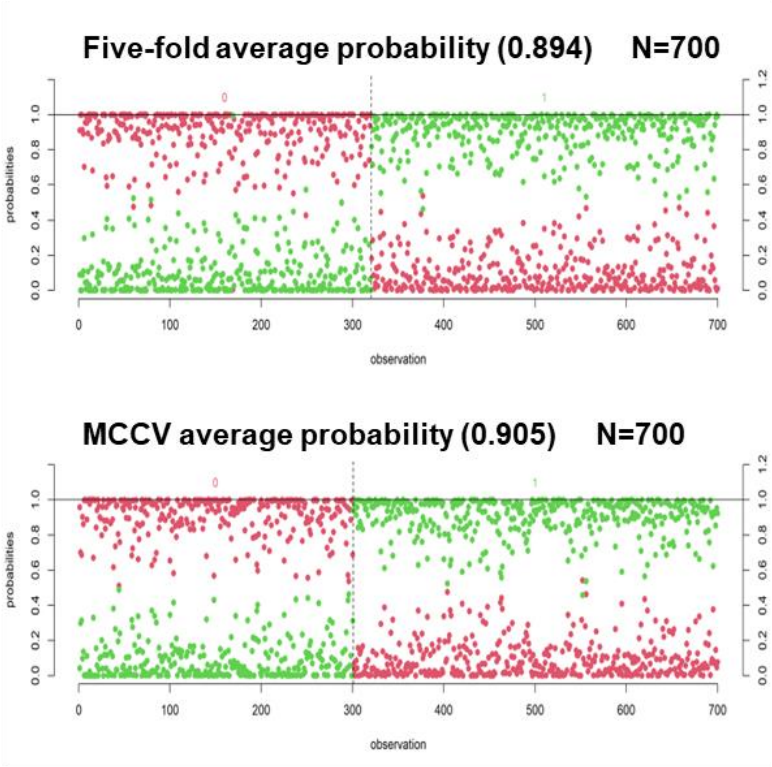
		predicted 0	predicted 1
true	0	315	5
	1	1	379

MCCV

number of missclassifications: 1
missclassification rate: 0.001
sensitivity: 0.998
specificity: 1

		predicted 0	predicted 1
true	0	300	0
	1	1	399

E.)



F.)

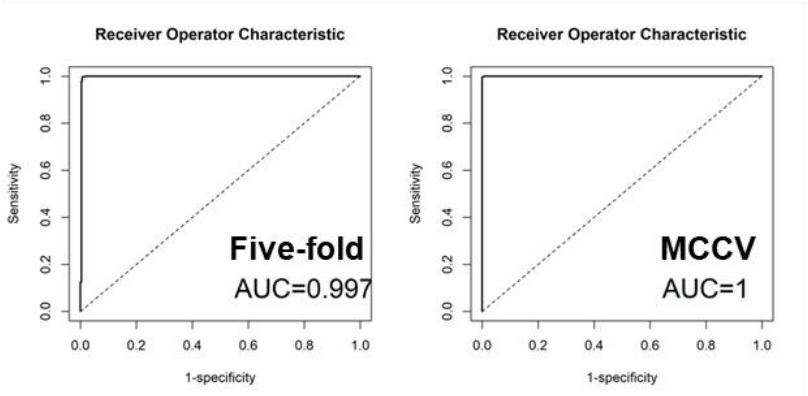


Figure 3.23. Comparison of the performance of the classification models generated by FFCV and MCCV re-sampling methods. Quantification of performance via sensitivity, specificity, misclassification rate and confusion table of the classification model in **A.)** TMS and **D.)** Sham**.** The rows in the confusion matrixes show the true positive and true negative values, and the columns present the predicted category. The average probability of correct classification plots in **B.)** TMS and **E.)** Sham. Each dot represents a subject in the validation set from each round of iteration during FCCV and MCCV re-sampling. N refers to the number of validated subjects in each method. The colour denotes the evaluation status, the correct classification is in green, and the incorrect classification is in red. The ROC curve (receiver operating characteristic curve) details the rate of true positives against false positives over the range of possible prediction outcomes in **C.)** TMS and **F.)** Sham.

C.3.7.7. Comparison of epilepsy-associated variables between TMS and SHAM-TMS datasets

Variable is the power ratio per frequency bin computed from the electroencephalogram recorded in the 21 recording channels at 18 stimulation sites (recording points). As defined in material and methods (section C.2.3.4), a recording point (RP) is defined as a specific recording channel during TMS stimulation at a specific area.

A variable is dubbed an epilepsy-associated variable if the means of the epilepsy and non-epilepsy two populations differ at a single frequency bin level. Variables in an array of sequential frequency bins ($n \geq 2$) in a recording channel at a stimulation site identified as epilepsy-associated variables are likely to represent a differential peak in the power ratio curve (Figure 3.24 and Table 3.7).

To understand how the TMS aids the epilepsy classification, the distribution of the epilepsy-associated variables, the topographic localisation and spectral frequency characteristics between TMS and Sham-TMS will be addressed.

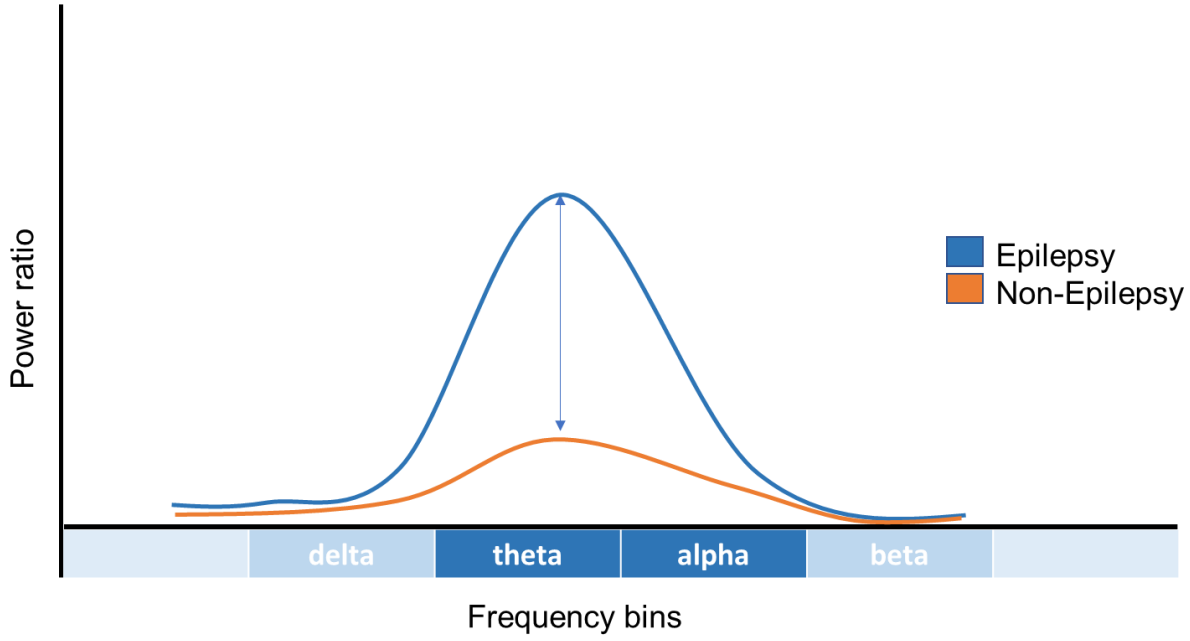


Figure 3.24. Schematic representation of epilepsy-associated variable.

Two variables with two sequential frequency bins in the epilepsy cohort

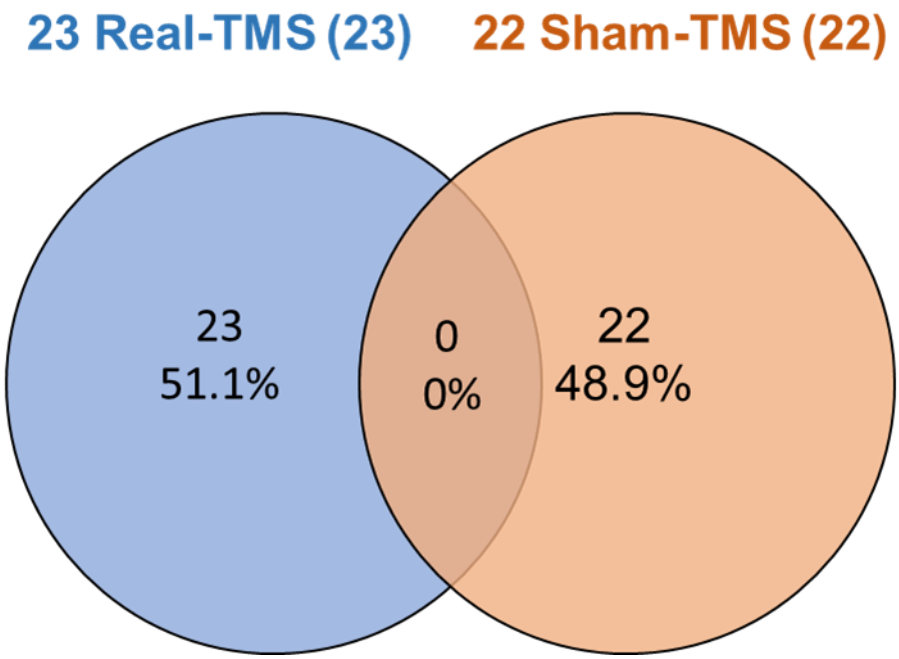


Figure 3.25. Epilepsy associated variables were distinct in the TMS and Sham-TMS datasets.

LIST OF SELECTED VARIABLES						
TMS Dataset (23)				Sham-TMS Dataset (22)		
SP	RC	Frequency		SP	RC	Frequency
C3	C3	δ		C3	A2	β
C3	C3	α		C3	F4	β
C3	C3	β		C4	F4	β
C3	F8	θ		CzA	A1	γ
C3	F8	γ		CzB	Fz	α
C4	C4	θ		F3	T5	α
C4	C4	α		F4	T6	δ
C4	P4	α		FzA	C3	γ
CzA	P4	θ		FzB	T5	α
CzB	Fz	θ		P4	Pz	γ
F3	A1	γ		P4	Fp2	θ
FzB	C3	γ		P4	Cz	β
FzB	Fp2	θ		P4	C3	β
FzB	Fp2	γ		P3	C3	δ
O1	A2	δ		P3	C3	β
O2	C3	α		P3	Cz	β
PzA	C4	α		P3	P3	δ
PzA	F3	γ		P3	T6	θ
PzA	T5	δ		PzB	P4	δ
PzB	P4	β		T3	A2	γ
T3	O1	β		T4	A2	γ
T3	O1	γ		T6	A1	α
T4	C3	α				

Table 3.7. The list of epilepsy-associated variables in the TMS and Sham-TMS datasets. Each raw in the table represents a variable. SP, stimulation point; RC, recording channel; FB, frequency band: α, Alpha (≥8, <14 Hz); β, Beta (≥14, ≤30 Hz); θ, Theta (≥4, <8 Hz); δ, Delta (<4 Hz); γ, Gamma (>30 Hz). Variables with sequential frequencies bins (n ≥2) in the same recording channel and stimulation point are labelled in blue (TMS). The colour gradient indicates the spectrum with a brighter tone for lower frequency (alpha) and a darker tone for higher frequency band (beta, gamma).

A.)

97	Fp2	Fp1	F8	F4	Fz	F3	F7	A2	T4	C4	Cz	C3	T3	A1	T6	P4	Pz	P3	T5	O2	O1	RC
C3			θ γ									α–β δ										2
C4										θ-α						α						2
CzA																θ						1
CzB					θ																	1
F3														γ								1
F4																						
FzA																						
FzB	θ γ											γ										2
O1								δ														1
O2												α										1
P3																						
P4																						
PzA						γ				α									δ			3
PzB																β						1
T3																					β-γ	1
T4												α										1
T5																						
T6																						
SC	1		1		1	1		1		2		4		1		3			1		1	

B.)

92	Fp2	Fp1	F8	F4	Fz	F3	F7	A2	T4	C4	Cz	C3	T3	A1	T6	P4	Pz	P3	T5	O2	O1	RC
C3				β				β														2
C4				β																		1
CzA														γ								1
CzB					α																	1
F3																			α			1
F4															δ							1
FzA												γ										1
FzB																			α			1
O1																						
O2																						
P3											β	δ β			θ			δ				4
P4	θ										β	β					γ					4
PzA																						
PzB																δ						1
T3								γ														1
T4								γ														1
T5																						
T6														α								1
SC	1			2	1			3			2	3		2	2	1	1	1	2			

Table 3.8. Table showing the relevant recording points (RP) and frequencies involved in **A.)** the 23 variables selected in the TMS and **B.)** the 22 variables selected in the Sham-TMS experiments. The first column indicates the 18 stimulation channels, and the first row the 21 recording channels. The 378 squares represent the recording points. The colour filled squares represent the recording points containing variables in close proximity frequency bin ranges in the TMS (blue) dataset. The RC column indicates the total number of recording channels with variables at a given stimulation area. The SC row indicates the total number of stimulation channels with variables at a given recording channel. The colour denotes the topographic localisation of the stimulation channels; parasagittal channels - orange, midline channels – yellow, temporal channels – green, posterior – white. FB; α, Alpha (≥8, <14 Hz); β, Beta (≥14, ≤30 Hz); θ, Theta (≥4, <8 Hz); δ, Delta (<4 Hz); γ, Gamma (>30 Hz).

C.3.7.8. Topographic comparison of epilepsy-associated features between TMS and Sham.

The intersection between the 18 stimulation areas and the 21 recording channels defined the 378 recording points (RP) where the selected variables resided. Of these 378 recording points, merely 17 (4%) and 21 (5%) were related to epilepsy classification and displayed a distinct and non-overlapping pattern in the TMS and Sham-TMS datasets, respectively (Table 3.9).

Examining through the topographic map, the TMS stimulation over the anterior and posterior midline (FzB, PzA) elicited a higher number of recording channels containing variables in the TMS than in the Sham-TMS dataset. The TMS over the parietal areas (P3, P4) did not result in any recording channel affiliated with epilepsy; in contrast to the Sham, in which the P3 and P4 stimulation channels showed beta frequency variables recoded at Cz and C3, gamma frequency variables recorded at PZ and delta-theta frequency variables recorded at C3, P3, T6 and Fp2. The TMS stimulation at T3 resulted in beta frequency variables over the recording channel O1.

The TMS stimulation over the central parasagittal (C3, C4) areas showed variables in different frequency ranges over three different recording channels: C3 (in the alpha-beta and delta bands), C4 (in the theta-alpha bands) and F8 (theta and gamma bands). While with the C3/C4 Sham stimulation, variables in the beta band appeared on the F4 and A2 recording channels.

Upon stimulation of the left frontal (F3), central midline (CzA/CzB), central parasagittal (C3, C4) and mid temporal areas (T3, T4), there was no difference in the number of recording channels (RC) containing sequential variables between the TMS and the Sham. The differences resided in the localisation and frequency band (FB) of these

variables. The stimulation at F3 resulted in variables in different frequency ranges, recorded on different channels depending on TMS (gamma band, A1) or Sham (alpha band, T5) stimulation. Moreover, the stimulation at T4 revealed major differences between TMS and Sham in the frequency spectrum, with the TMS showing variables in the alpha band recorded in the C3 channel while the variables recoded at A2 with Sham were in the gamma range (Table 3.8).

The TMS stimulation in the midline showed mixed results, depending on the anterior to posterior topographic localisation and the stimulation modality A/B. A higher recording channel count was seen at the posterior sagittal stimulation with modality A (PzA), where the TMS generated variables in the alpha, gamma and delta frequencies over C4, F3 and T5 respectively while only one variable in the beta band was provoked with modality B. The pattern was inversed at the anterior midline stimulation areas (Fz) as the TMS stimulation modality A did not elicit any variables while gamma and theta band variables appeared in the recording channel C3 and Fp2 with Fz stimulation in modality B. Also, the Fz stimulation showed differences in the frequency spectrum between the TMS with gamma and theta frequency variables and the Sham, which showed gamma and alpha frequency variables recorded at the C3 and T5 recording channels with A or B stimulation modalities respectively (Figure 3.28). TMS did show theta band variables upon Cz stimulation, while the Cz-Sham showed variables in the gamma and alpha bands distributed in two different recording channels: A1 and Fz. There were no variables present after TMS stimulation over T5, T6, P3, P4, F4, neither after Sham stimulation over O1, O2 or after either TMS or Sham stimulation of T5.

When the location of the variables in the recording channels was studied in the Sham, the majority of these variables were detected in the mastoid regions (A1, A2), and some were grouped in the midline (Cz), central parasagittal (C3) and posterior

temporal recording channels. In the TMS dataset, the variables seemed to be widely distributed among the recording channels. Still, the majority of the variables were localised in the central-parasagittal (C3, C4), frontal (Fz, F3, Fp2) and posterior (P4, Pz, T5, O1) channels. Some variables were also detected in mastoid areas (A1, A2) (Figure 3.27A). Furthermore, most of the variables in the TMS were identified only with a specific area of stimulation for the majority of the recording channels. For example, the Fp2, Fz, F3 and T5 recording channels presented variables after stimulation over the midline (FzB, CzB, PzA/B) region; the F8 channel after stimuli in the left mid parasagittal (C3) region; the O1 channel after anterior temporal stimulation (T3) and the A1, A2 channels after stimuli in the anterior (F3) and posterior regions respectively. The C4, C3 and P4 were the only recording channels whose variables appeared after TMS stimulation over two or more different areas (Figure 3.27A).

Conversely, the 58% of the recording channels related to epilepsy classification had not had a one-to-one relationship with a particular stimulation area in the Sham experiment, and the variables appeared in the same recording channel regardless of the topography of the stimulation channel. For instance, the variables in the A2 recording channel were seen at any of the following stimulation landmarks C3, T3, and T4. The variables in the A1 recording channel were seen after simulation in CzA and T6. Also, the variables in the Cz recording channel were present at the P3 or P4 stimulation channels, the variables at C3 were seen with either FzA, P3 or P4 stimulation and the variables in F4 with stimulation landmarks at C3 and C4. The variables recoded over the posterior temporal regions followed the same trend with the variables in the T5 recording channel seen in the F3 and FzB stimulation landmarks and the variables in the T6 recording channel seen in the F4 and P3 stimulation regions. Only five exceptions, the remaining Sham recording channels

related to epilepsy classification, the Fp2, Fz, P4, Pz and P3 recording channels, had variables at particular stimulation areas, the P4, CzB, PzB, P4 and P3, respectively (Figure 3.27A). Therefore, the TMS experiment was more dependent on the TMS stimulation area and modality than the Sham for variable selection.

C.3.7.9. Frequency spectrum comparison of epilepsy-associated features between TMS and Sham.

Of the total 23 epilepsy-associated variables identified in the TMS, 12 (52%) were in the alpha (6) and gamma (6) frequency bands, 5 (21%) were in the theta frequency band and the remaining 26% were in the beta (3) and delta (3) frequency range. In the Sham experiment, only two of the total 22 epilepsy-associated variables belonged to the theta band (2%), while the majority were scattered in the beta (32%)-gamma (23%) followed by the alpha-delta (26%) frequency spectrum. Interestingly enough the epilepsy-associated variables in the beta and theta frequency bands display a distinctive distribution between the TMS and the Sham TMS datasets with a higher proportion of theta variables and a lower proportion of beta variables in the TMS in comparison to Sham (Table 3.10).

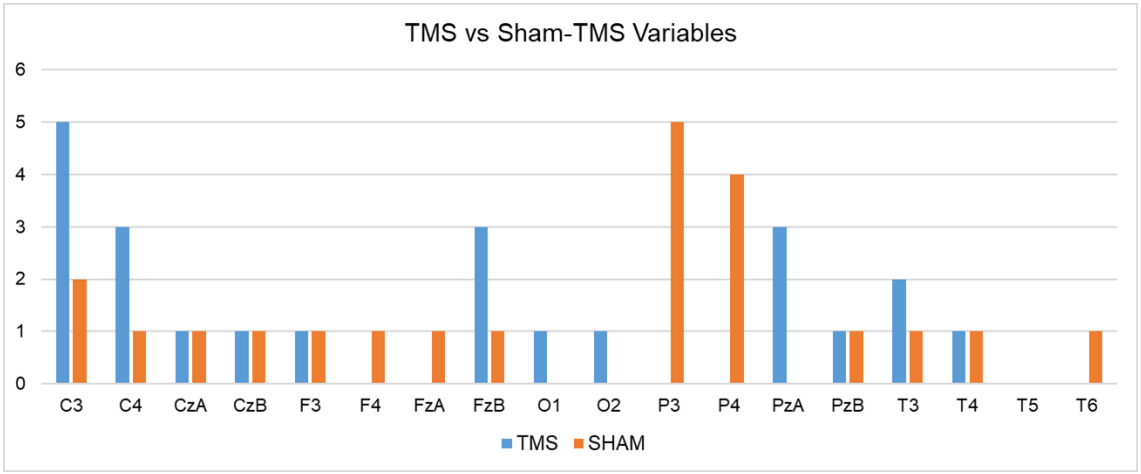
	TMS	Sham-TMS
Number of recording points containing sequential frequencies variables	3 (17.6%)	0 (0 %)
Number of recording points with exclusively non sequential frequencies variables	14 (82.4%)	21 (100%)
Total number of recording points with selected variables	17	21

Table 3.9. Summary of the recording points presenting epilepsy-associated variables.

Number of selected variables (total number)	TMS	Sham-TMS
α, Alpha	6 (26%)	4 (18.2%)
β, Beta	3 (13%)	7 (31.8%)
γ, Gamma	6 (26%)	5 (22.7%)
θ, Theta	5 (21.7%)	2 (9%)
δ, Delta	3 (13%)	4 (18.2%)
Total number of selected variables	23	22
Number of sequential variables	TMS	Sham-TMS
α, Alpha	2 (33.3%)	0 (0%)
β, Beta	2 (33.3%)	0 (0%)
γ, Gamma	1 (16.7%)	0 (0%)
θ, Theta	1 (16.7%)	0 (0%)
Total number of sequential variables	6	0

Table 3.10. Summary of the characteristics of the epilepsy-associated variables: frequency band and sequential pattern.

A.)



B.)

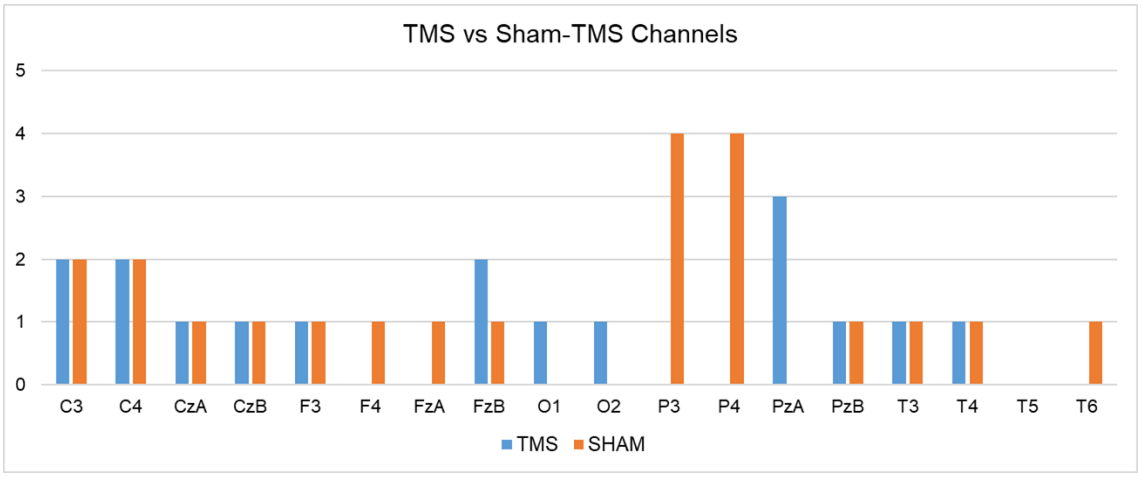
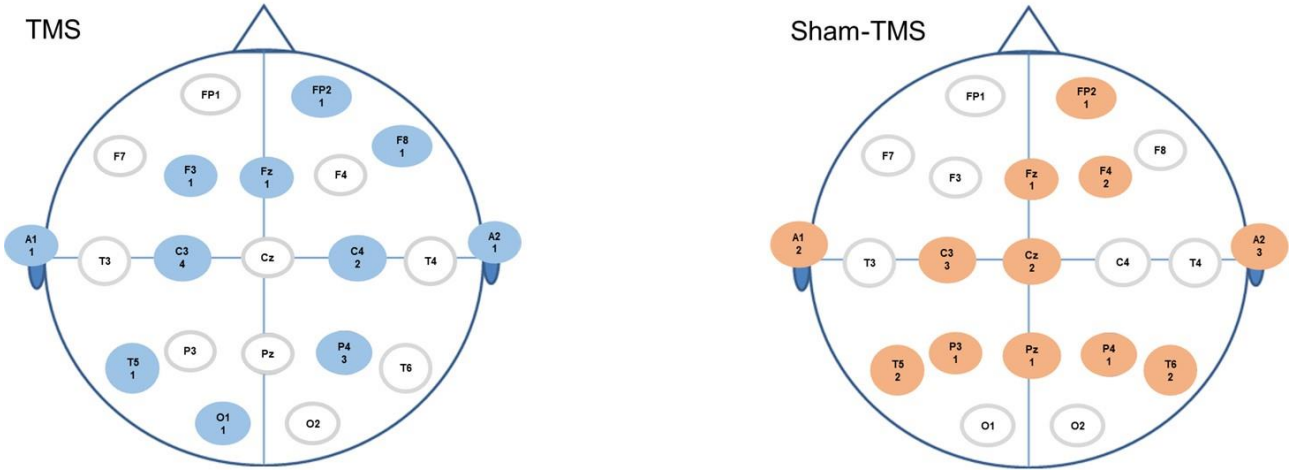


Figure 3.26. Topographic distribution of the epilepsy-associated variables in TMS and Sham experiments. The histogram plot illustrates **A.)** the number epilepsy-associated variables in a particular stimulation region with TMS (blue) or Sham (orange). The X-axis shows the stimulation areas, and the Y-axis the number of variables **B.)** the number of recording channels showing any epilepsy-associated variables when a particular region is stimulated with TMS or Sham. The X-axis shows the stimulation areas, and the Y-axis the number of recording channels.

A.)

Recording Channels – Number Stimulation points



B.)

Stimulation Points-Number of Recording Channels

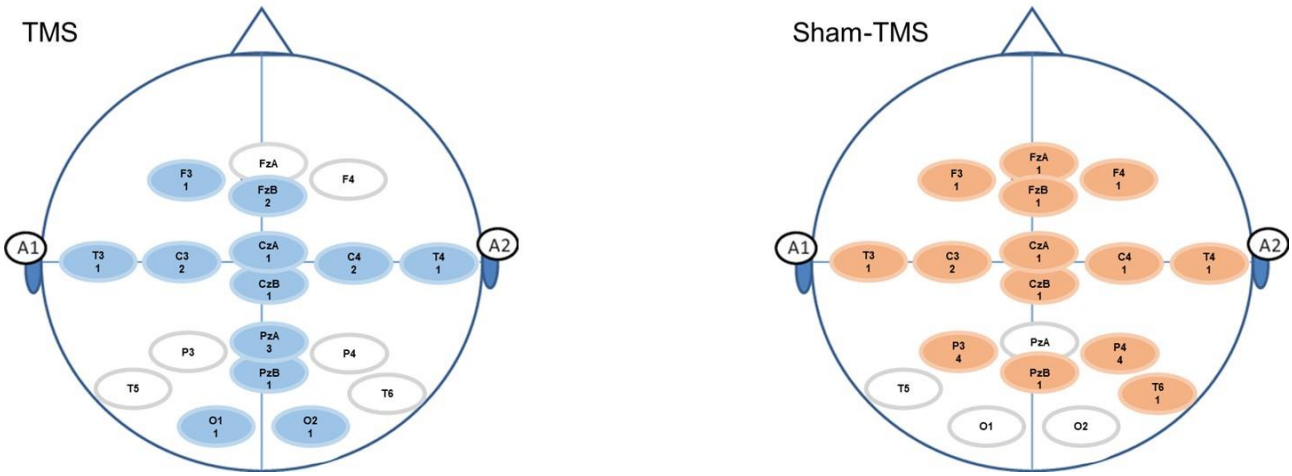
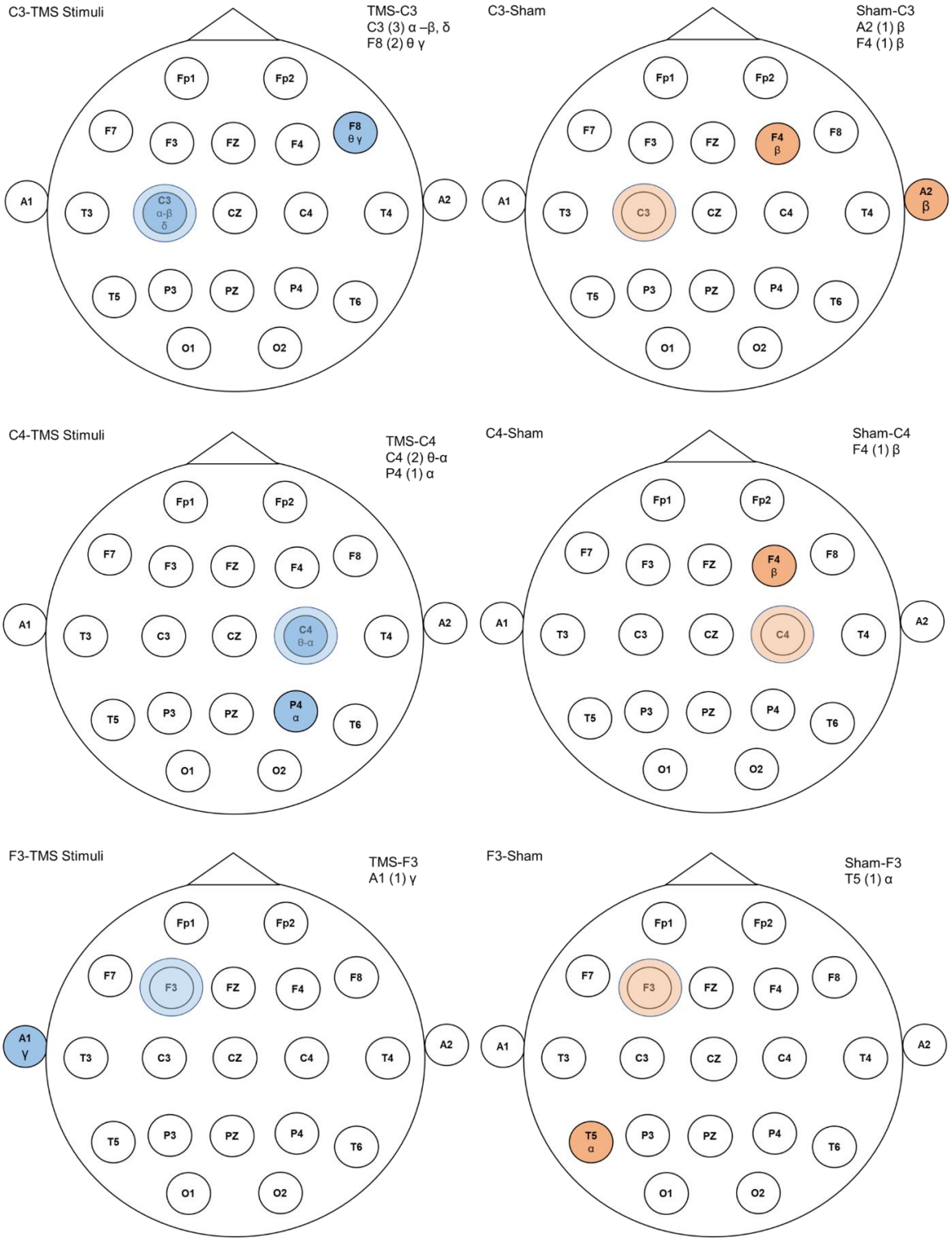
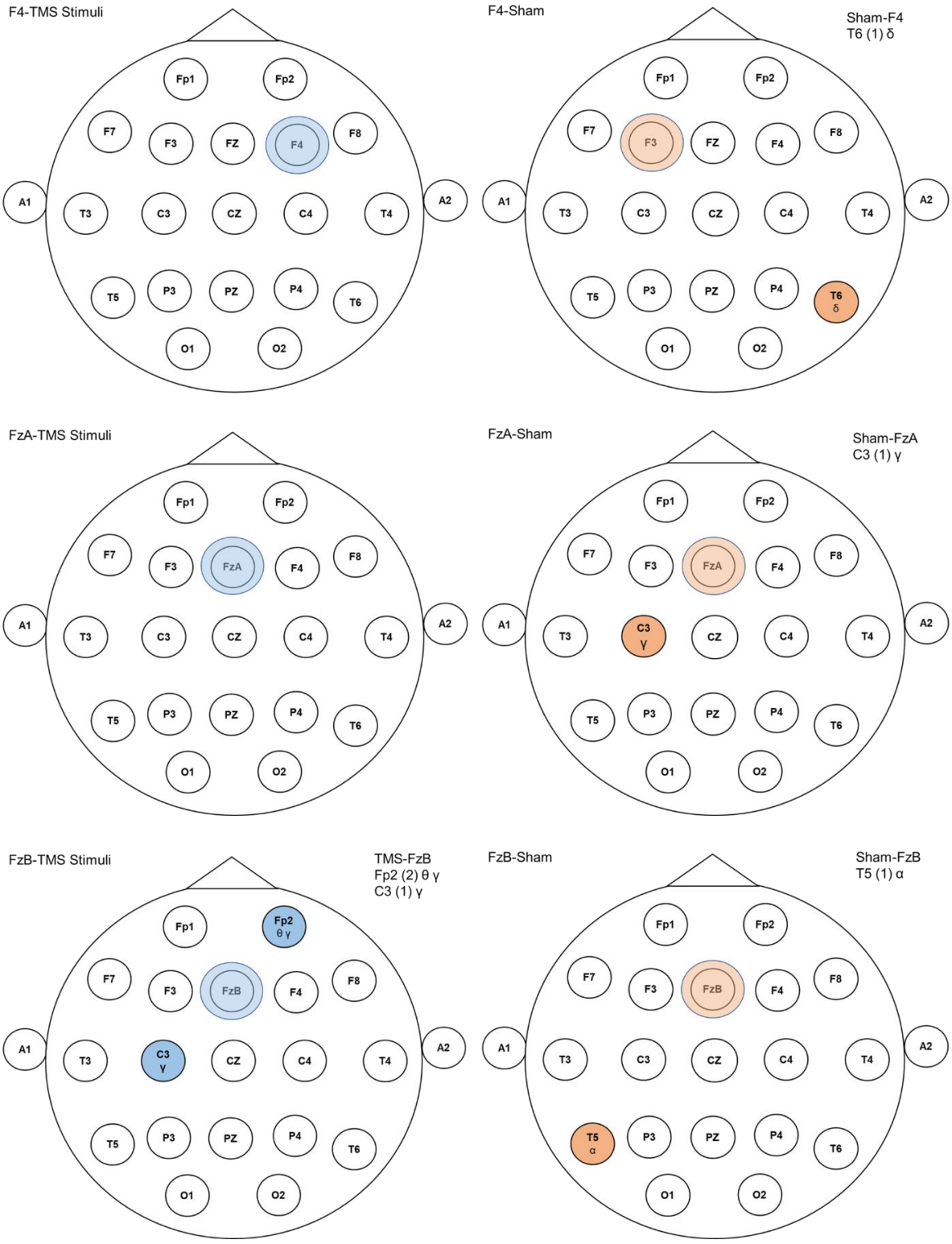
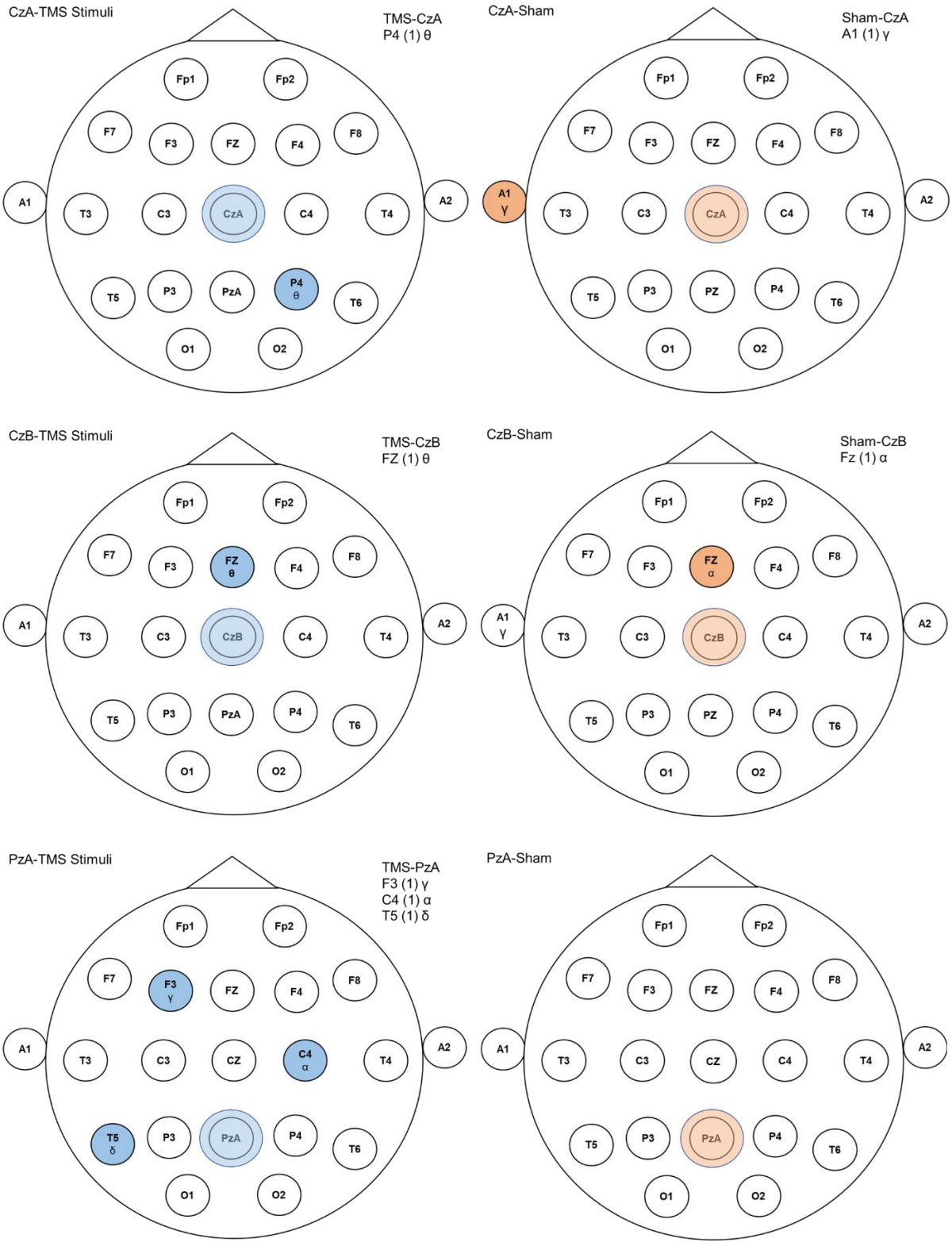


Figure 3.27. Topographic localisation of the stimulation and recording channels involved in the selection of epilepsy-associated variables in the TMS and Sham experiments. The head diagrams show the number of stimulation points (SP) and recording channels (RC) displaying epilepsy-associated variables in the TMS (blue colour) or Sham (orange colour) groups **A.)** The recording channels are represented by circles. The recording channel containing variables is coloured in orange for the Sham experiment and blue for the TMS experiment. The numbers inside the circles indicate the number of specific stimulation areas that generated the variables in the depicted recording point**. B.)** The ovals represent the stimulation areas (blue for TMS and orange for Sham), and the number inside each circle represents the number of recording channels that displayed variables upon the stimulation.


C3-TMS Stimuli
TMS-C3
C3 (3) α –β, δ
F8 (2) θ γ
C3-Sham
Sham-C3
A2 (1) β
F4 (1) β
C4-TMS Stimuli
TMS-C4
C4 (2) θ-α
P4 (1) α
C4-Sham
Sham-C4
F4 (1) β
F3-TMS Stimuli
TMS-F3
A1 (1) γ
F3-Sham
Sham-F3
T5 (1) α
Fp1
Fp2
F7
F3
FZ
F4
F8
A1
T3
C3
CZ
C4
T4
A2
T5
P3
PZ
P4
T6
O1
O2


F4-TMS Stimuli
F4-Sham
Sham-F4
T6 (1) δ
FzA-TMS Stimuli
FzA-Sham
Sham-FzA
C3 (1) γ
FzB-TMS Stimuli
TMS-FzB
Fp2 (2) θ γ
C3 (1) γ
FzB-Sham
Sham-FzB
T5 (1) α
Fp1
Fp2
F7
F3
FZ
F4
F8
A1
T3
C3
CZ
C4
T4
A2
T5
P3
PZ
P4
T6
O1
O2
FzA
FzB
T6 δ
C3 γ
Fp2 θ γ
T5 α


CzA-TMS Stimuli
TMS-CzA
P4 (1) θ
CzA-Sham
Sham-CzA
A1 (1) γ
CzB-TMS Stimuli
TMS-CzB
FZ (1) θ
CzB-Sham
Sham-CzB
Fz (1) α
PzA-TMS Stimuli
TMS-PzA
F3 (1) γ
C4 (1) α
T5 (1) δ
PzA-Sham
Fp1
Fp2
F7
F3
FZ
F4
F8
A1
T3
C3
CzA
C4
T4
A2
T5
P3
PzA
P4
T6
O1
O2
PZ
CzB
CZ
PzA


PzB-TMS Stimuli
TMS-PzB
P4 (1) β
PzB-Sham
Sham-PzB
P4 (1) δ
T3-TMS Stimuli
TMS-T3
O1 (2) β-γ
T3-Sham
Sham-T3
A2 (1) γ
T4-TMS Stimuli
TMS-T4
C3 (1) α
T4-Sham
Sham-T4
A2 (1) γ
Fp1
Fp2
F7
F3
FZ
F4
F8
A1
T3
C3
CZ
C4
T4
A2
T5
P3
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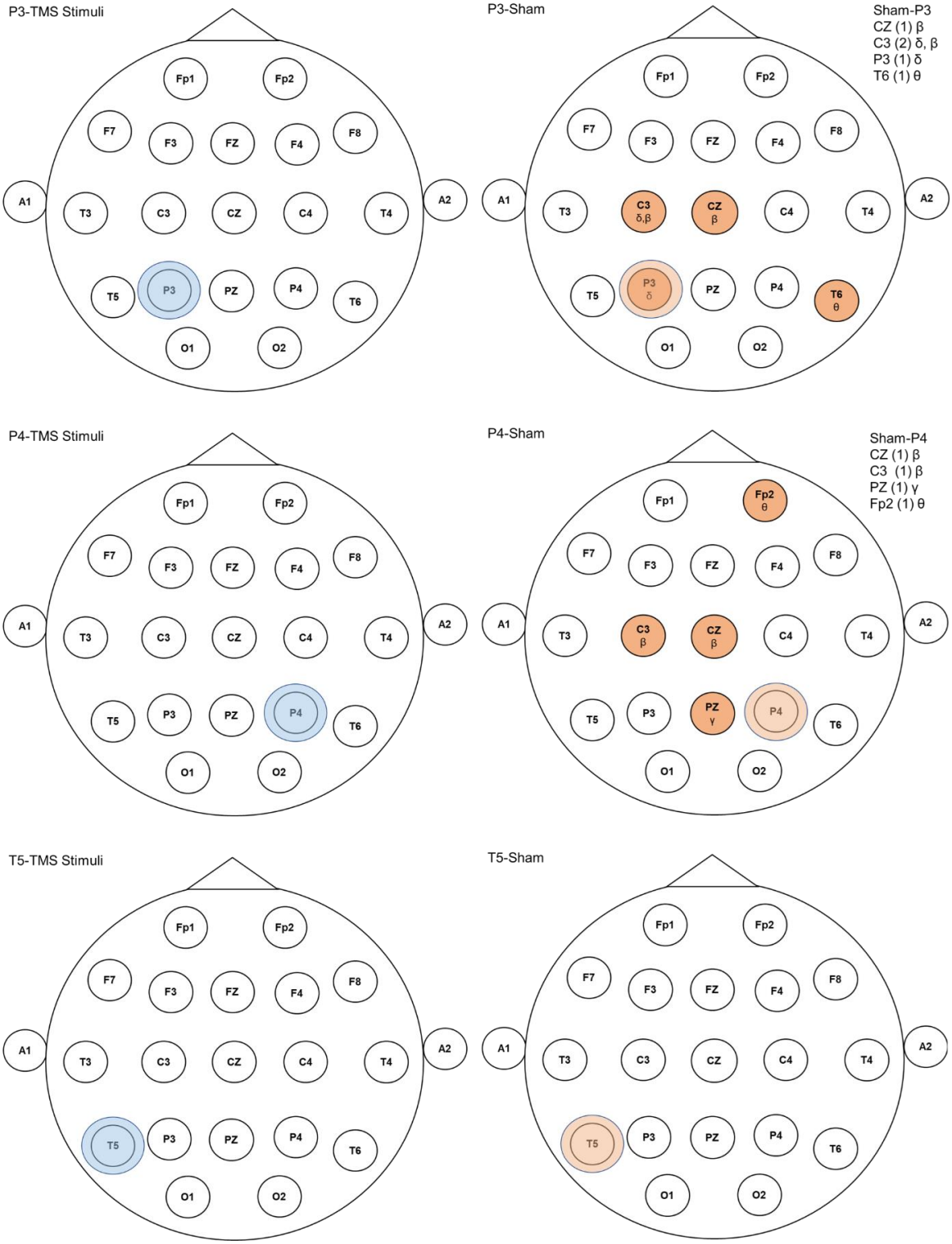
PzB
P4
β
P4
δ
O1
β-γ
C3
α
A2
γ


P3-TMS Stimuli
P3-Sham
Sham-P3
CZ (1) β
C3 (2) δ, β
P3 (1) δ
T6 (1) θ
P4-TMS Stimuli
P4-Sham
Sham-P4
CZ (1) β
C3 (1) β
PZ (1) γ
Fp2 (1) θ
T5-TMS Stimuli
T5-Sham
Fp1
Fp2
F7
F3
FZ
F4
F8
A1
T3
C3
CZ
C4
T4
A2
T5
P3
PZ
P4
T6
O1
O2


T6-TMS Stimuli
T6-Sham
Sham-T6
A1(1) α
O1-TMS Stimuli
TMS-O1
A2 (1) δ
O1-Sham
O2-TMS Stimuli
TMS-O2
C3 (1) α
O2-Sham
Fp1
Fp2
F7
F3
FZ
F4
F8
A1
T3
C3
CZ
C4
T4
A2
T5
P3
PZ
P4
T6
O1
O2

Figure 3.28. Summary of the characteristics of the epilepsy-associated sequential variables: topographic localisation, number and frequency spectrum. The double circles represent the stimulation areas (blue for TMS and orange for Sham), the circles represent the recording channels (RC), and the Greek characters inside each circle represent the frequency band of the selected sequential variables present in the recording channel (RC). The legend on the left-up corner of each head figure shows the stimulation channels (SC), TMS or Sham. The legend on the right-up corner shows the recordings channels (RC) displaying sequential variables, the number of sequential variables selected in each RC (the number inside the brackets) and the variable frequency range: α, alpha (≥8, <14 Hz); β, beta (≥14, ≤30 Hz); δ, γ, Gamma (>30 Hz).

3.7.10. Prediction of the machine learning classification models when applied to a new independent cohort

The 22 variables Sham model has a low sensitivity (below 70%) to predict epilepsy.

The specificity is also low in both MCCV and FFCV (Table 3.11).

Five-fold CV

number of missclassifications: 14
missclassification rate: 0.378
sensitivity: 0.695
specificity: 0.5

		predicted	
		E	NE
true	E	16	7
	NE	7	7

MCCV

number of missclassifications: 17
missclassification rate: 0.459
sensitivity: 0.521
specificity: 0.571

		predicted	
		E	NE
true	E	12	6
	NE	11	8

Table 3.11. Performance indicators of the 22 variables Sham SVM classifier to predict epilepsy in the independent cohort. Contingency tables showing the sensitivity and specificity and misclassification rate of the 22 variable Sham SVM-classification model to predict epilepsy in the independent dataset based on FFCV and MMCV methods. The rows in the confusion tables show the predicted category, and the columns present the true clinical classification (C.C.); E, epilepsy; NE, no epilepsy.

The 23 variables TMS classifier was a better prediction model than Sham, showing a higher sensitivity (83%) to predict epilepsy. Regrettably, the specificity remained low (Table 3.12).

Five-fold CV

number of missclassifications: 14
missclassification rate: 0.378
sensitivity: 0.826
specificity: 0.285

		predicted E	predicted NE
true	E	19	10
	NE	4	4

MCCV

number of missclassifications: 19
missclassification rate: 0.513
sensitivity: 0.608
specificity: 0.287

		predicted E	predicted NE
true	E	14	10
	NE	9	4

Table 3.12. Performance indicators of the 23 variables TMS SVM classifier to predict epilepsy in the independent cohort. Contingency tables showing the sensitivity and specificity and misclassification rate of the 23 variable TMS SVM-classification model to predict epilepsy in the independent dataset based on FFCV and MMCV methods**.** The rows in the confusion tables show the predicted category, and the columns present the true clinical classification (C.C.); E, epilepsy; NE, no epilepsy.

CHAPTER 4. DISCUSSION

The discussion is divided into three different sections to focus separately on the TMS protocol optimisation, the visual features of the TMS-EEG and the quantitative analysis of the TMS-EEG recordings.

This TMS–EEG study aims to design a TMS stimulation protocol suitable to investigate cortical excitability in clinical settings and to explore the diagnostic potential of this TMS-EEG protocol in a cohort of patients with newly diagnosed epilepsies.

C.4.1. TMS-EEG recording protocol optimisation

The TMS–EEG protocol optimization intends to design a TMS diagnostic protocol that would be both tolerable for the patients and effective to reveal the EEG features associated with epilepsy. Thus, a brief comment on the important aspects of the protocol follows.

C.4.1.1. Appropriate stimulation intensity and selection of the motor threshold (MT) calculation protocol.

The importance of choosing an adequate TMS stimulus intensity is that different subjects may be differentially sensitive to the TMS stimulation due to inter-individual variability reflecting idiosyncratic cortical excitability and anatomical differences such as the skull to cortex distance or the corticospinal tract's structural characteristics. Different methods of MT calculation have been reviewed in the literature (see Introduction, Chapter 1, Section C.1.3.2.2.). Awiszus' threshold-hunting algorithm was implemented in this study to measure the MT of the participants. The reason for selecting this adaptative method is that it allows a faster MT calculation because it requires a smaller number of stimuli than the relative frequency methods without

compromising accuracy. The IFCN and the literature support adaptative threshold hunting protocols as a more efficient tool for MT estimation and as a preferable option over relative frequency methods (Awiszus, 2011, Qi et al., 2011, Groppa et al., 2012, Awiszus, 2003, Mishory et al., 2004, Silbert et al., 2013). Also, the maximum likelihood algorithm program is freely available by Awiszus and Borkardt 2011 (Motor Threshold Assessment Tool, version 2.0: htpp://www.clinicalresearcher.org/software), making it easily accessible and convenient for the study.

The experience with Awiszus' method in this project is that it is a safe, efficient and precise protocol for motor threshold calculation.

Handedness was not assessed prior to the motor threshold calculation. Although a lower threshold has been reported for the dominant hemisphere, and some authors advocate the documentation of handedness (Macdonell et al., 1991, Triggs et al., 1994, Triggs et al., 1999), this interhemispheric difference, if present at all, is physiologically minimal to the extent that in some studies side to side MT comparison is implemented as a diagnostic tool in mono hemispheric lesions as it is assumed that a significant interhemispheric MT variation is suggestive of a lesion (Traversa et al., 1998).

C.4.1.2. Adequate type of TMS coil.

Single-pulse systems commonly have a circular 90mm diameter coil. Most circular coils have good penetration to the cortex, but they lack focality as single coils induce fields over a wider area. Conversely, double coils induce more localised electrical fields. Both options have a priori advantages and disadvantages for the diagnosis of epilepsy. For the purpose of the present project, the stimulation of a wider cortical area with a single round coil was favoured as the best protocol to induce late EEG

responses to TMS in epilepsy patients. The properties of the circular coil were useful for this study as the target for stimulation was uncertain, and the circular coil induced an electrical current in a large volume of brain tissue that was homogeneously stimulated in a non-focal fashion. The figure of eight coil stimulates a brain region more selectively, and it would be more suited for the study of localized regions in focal epilepsies. On the other hand, the circular coil may be more efficient in terms of exploring diffuse cortical hyperexcitability in this epilepsy cohort from the adult first seizure clinic comprised of both generalized and focal epilepsies. Furthermore, the use of a circular coil for TMS stimulation in this protocol allows to generate an equivalent electrical field (EF) over similar EEG positions in both hemispheres; while, with the figure of eight coil, the direction of the induced EF over the target areas is easily modified by minimal changes in the coil orientation and angle (see section C.1.3.1.4).

C.4.1.3. Selection of the TMS stimulation positions in the scalp and the effective number of pulses per location.

The most commonly implemented single pulse TMS-EEG protocols for the study of epilepsy consist of the stimulation over a specific area of the brain; generally, the motor cortex (M1) or the dorsolateral prefrontal cortex (DLPFC), to evoke TEPs.

In this study, a different approach was taken to more widely stimulate the cortex to evoke TMS late responses based on previous results obtained in long-standing focal epilepsies (Valentin et al., 2008). The EEG 10-20 international standard electrode positions were used as landmarks to position the TMS coil, with the exception of the Fp1, Fp2, F7 and F8 positions. The decision to remove these positions from the stimulation protocol was based on the little information that was extracted from these

positions in the initial assessment made with a small cohort of volunteers and patients. This was due to the fact that the TMS stimulation over these areas was more uncomfortable, generated more artefacts and in some cases had to be abandoned due to pain before the completion of the minimum number of pulses required to generate reliable TMS-evoked responses.

The number of TMS pulses delivered at any given stimulation area was 15. This was considered an adequate number of stimuli, allowing some scope for the rejection of up to five artefactual epochs prior to the analysis, obtaining a minimal of 10 epochs. The minimal number of pulses required to show clear, robust and reliable repetitive TMS-EEG responses after average was estimated to be 10. This estimate was based on previous work using a figure of eight coil for the study of patients with focal epilepsy, which suggested between 8-15 pulses per scalp position as an adequate number of stimuli to show repetitive responses to TMS after averaging (Valentin et al., 2008).

C.4.1.4. Report of unexpected side effects of TMS-EEG.

The expert opinion states that the risk of seizures during single-pulse TMS (spTMS) is extremely low. To my knowledge, TMS associated seizures in control volunteers undergoing single-pulse TMS have not been reported, and the risk of TMS associated seizures in epileptic patients had been reported to be within 0.0 to 2.8% for spTMS (Schrader et al., 2004).

In keeping with the literature on single-pulse TMS, no unexpected side effects of TMS-EEG were observed in this study. The spTMS-EEG has proven to be a safe and, for most patients, tolerable procedure, as supported by the results of the feedback questionnaire completed by the participants after the TMS-EEG study (see results chapter, Section C.3.5).

C.4.2. Qualitative analysis: Visual TMS-EEG analysis to identify the epileptogenic trait

To evaluate the diagnostic effectiveness of the TMS-EEG study's protocol and the visual analyses, the following is considered:

C.4.2.1. Motor Threshold (MT)

TMS-EMG studies assessing the MT in epilepsy show contradictory results in the literature, with some studies showing a reduced MT in untreated GGE in comparison to controls while others show no differences between epilepsy and controls. In this study, the mean MT values were significantly lower in drug-naive epileptic patients than in non-epileptic patients (see Results chapter 3, Section C.3.2). However, there were no differences between epilepsy patients treated with antiepileptic drugs (AED) and no-epileptic subjects, maybe due to the increase of the MT in patients treated with AEDs reported in the literature (see Introduction, section C.1.4.2.).

C.4.2.2.TMS as an activation technique of interictal epileptiform discharges (IEDs) in the EEG

Other groups have implemented TMS as an activation technique to trigger or evoke IEDs (Kimiskidis et al., 2017, Schuler et al., 1993). Schuler evaluates the effectiveness of TMS in focal epilepsy as an activation technique in comparison to over-breathing, a well-established manoeuvrer for activation of epileptiform activity in the EEG. In Schuler's study, only three out of ten cases showed activation of the EDs with TMS stimulation at 0.05 to 0.3 Hz, and in three out of ten cases, TMS caused a reduction in the number of the IEDs. TMS-induced epileptiform discharges (EDs) are defined by

some authors as interictal and ictal patterns containing spike and wave complexes with a zero-time lag following magnetic stimulation, with a first spike appearing on the descending slope of the N100 wave (Kimiskidis et al., 2017). In Kimiskidis et al.'s experience in patients with GGE, the circular coil was more efficient than the figure of eight to induce EDs. Also, the biphasic pulse waveform was more effective than the monophasic pulse, and the paired pulse stimuli provided better results than the single-pulse paradigm in inducing EDs. Although the single pulse paradigm with a circular coil and a monophasic pulse used in the present study was not the optimal protocol to activate EDs in GGE based on the literature and the pulses in this study were delivered at a lower frequency (0.2Hz) than in the previously mentioned studies (ranged between 0.3 to 4Hz), it should be considered that the objective of the present study was to evoke and assess TMS-EEG rhythms associated to epilepsy but not necessarily to implement a protocol for provocation of EDs. Whereas, in two GGE patients in this cohort, occasional EDs appear in close proximity (zero time-lag) to the TMS artefact (100-200 msec). These patients also had IEDs in the routine EEG and non-induced spontaneous IEDs in the TMS-EEG. So, the possibility of these EDs being just coincidental to the TMS pulse rather than generated by it has to be considered. Overall, in my sample, the number of IEDs did not increase significantly in the TMS-EEG study in comparison to the baseline EEG. Neither the NSDs were activated by TMS in the epilepsy group. Interestingly enough, the results show a tendency to a reduction in the number and duration of the of NSDs in the TMS-EEG study in the non-epileptic group but not in the epileptic patients. Although the numbers were small to establish sound inferences, it can be argued that the trend of the reduction in the number and duration of NSDs may be related to the variability of the state of alertness or arousal mechanisms during the TMS, especially considering that

the participants were sleep-deprived at the time of the study. It is of interest the fact that the same trend of NDSs reduction with TMS was not seen in the epilepsy group. As an excessively enhanced response to single-pulse TMS stimulation after sleep deprivation has been reported in epilepsy patients (Del Felice et al., 2011), a possible hypothesis for the immutable persistence of the NSDs during the TMS-EEG would be that the epileptic patients recover more slowly from the enhanced excitability stage generated by sleep or that the impact of the state of arousal or sleep deprivation on the TMS modulatory effect of ongoing EEG rhythms would differ between epileptic and non-epileptic subjects (Del Felice et al., 2011). This may open new avenues for further studies looking into the TMS modulation of nonspecific EEG rhythms and TMS modulation of arousal related EEG background changes as an indirect biomarker of epileptogenicity or non-epilepsy related idiosyncratic responsiveness to TMS in VVS or PNES.

C.4.2.3.TMS-EEG evoked late responses as a biomarker of epileptogenicity

The importance of the late responses as a biomarker of epileptogenicity in long-standing focal epilepsy has been suggested in the study by Valentin et al. at KCL (Valentin et al., 2008). In this study, the late responses are divided into delayed responses (DRs) and repetitive responses (RRs):

Delayed responses (DRs)

In the literature, the DRs are described as spikes or sharp waves resembling IEDs and appearing between >100 msec and < 1-second post-TMS stimulation. The DRs are not strictly time-locked to the TMS stimulation as they do not always follow the TMS pulse (occurrence rates were reported between 35% and 100%, depending on the patient and stimulation site). The time-lapse between the TMS pulse and the DRs is

variable, and, in contrast to the TMS-induced EDs described by Kimiskidis, the time-lapse can stretch up to 1 second.

As morphologically, the DRs may be indistinguishable from spontaneous IEDs; the DRs are most precisely defined by a quantitative criterion, considering that the EDs appearing after TMS pulses could be defined as DRs evoked by TMS stimulation if the number of discharges during the second following stimulation was greater than the number discharges during the second prior to stimulation. Following this criterion, the DRs only appeared in 20% of the focal epileptic patients with long-standing disease and only when stimulating over the epileptogenic focus (Valentin et al., 2008). In this study, the DRs appear only in epileptic patients, specifically in patients with GGE, and they were not seen in focal epilepsy. Possible explanations for the lack of DRs in the sample of patients with focal epilepsy in this study would be the less localised stimulation with the circular coil (Deng et al., 2013) or the shorter temporal course of the disease of the new-onset focal epilepsy. In this study, the DRs were seen in a slightly lower percentage in the GGE participants (15%) than in the focal epilepsy cohorts reported in the literature (20%). The DRs in this study were accompanied by other abnormalities in the baseline EEG and/or TMS-EEG, therefore not adding particularly useful information for diagnosis. However, in a particular case, the delayed responses contributed to reinforcing the TMS-EEG classification of epilepsy in a patient with a normal baseline EEG and final diagnosis of GGE. In this patient, the DRs were a clear abnormal finding in the TMS-EEG in the absence of overt IEDs in the baseline EEG study. Also, in this patient, the presence of DRs further supported the final classification of RRs reported by the third observer after an initial inter-observer disagreement regarding the presence of RRs. This finding also suggests that in particular scenarios, such as a false negative routine EEG, the DRs may

increase the sensitivity of the TMS-EEG to detect epilepsy in comparison to the EEG alone (Results Chapter 3, Figures 3.9 and 3.10). The DRs are highly specific in this cohort as, contrary to other TMS-EEG abnormal features such as the RRs, DRs are exclusively recorded in the epileptic patient and not in the VVS or PNES.

Repetitive responses (RRs)

As stated in the literature, the second type of late response, the repetitive responses (RRs), are characterised by their clear interhemispheric asymmetry as the RRs are only present in the affected hemisphere of patients with focal epilepsy.; therefore, the presence of background amplitude changes following TMS pulses (RRs) in a particular area could be compared to the imperceptible or much more reduced changes following TMS in the same position in the contralateral hemisphere.

In this project, the first observation was the moderate inter-observer agreement in the assessment of the RRs. This fact may be due to the elusive nature and distinct morphological characteristics of the RRs in our sample. The asymmetrical features in the RRs may not be as clearly present to the observers resulting in the inter observers' discrepancies in this study. Also, in some instances, the characteristically expected amplitude change (approximately 50% increment) following the TMS pulse in comparison to the pre-TMS stimulation background was less obvious in this cohort, further contributing to the inter observers' variability.

On the literature, RRs are reported to be significantly associated with the presence of epilepsy, being highly specific (100% specificity). In this sample, the recording of RRs alone was also significantly associated with the presence of epilepsy, but the specificity was lower (85%). A possible explanation for the lower specificity in the present study is that the RRs in epileptic patients were compared against non-epileptic

patients suffering from VVS or PNES instead of against healthy controls. It may be speculated that those VVS and/or PNES patients suffer from an underlying hyperreactivity to TMS stimulation unrelated to epilepsy. To my knowledge, there is no literature regarding TMS-EEG generated rhythms in VVS or patients with PNES only, and the study of TMS-EEG features in VVS patients may be an interesting new avenue for research. Other possible explanations for the lower specificity of the RRs in the present sample are:

- In generalised epilepsies with more diffuse anomalies of cortical excitability (Badawy et al., 2007), it would be more difficult to accurately detect RRs with visual assessment than in long-standing focal epilepsies. The background difference following TMS stimulation or asymmetric increment in the background after TMS pulse, which is one of the landmarks of the RRs, is not as marked in this study. As the stimulation parameter, protocols and analysis methodology for RRs were in keeping with the literature; it may be argued that the idiosyncrasy of the sample influenced the findings. The only methodological difference in this work was the use of a circular coil instead of a figure of eight coil that was favoured in other studies. The circular coil ensures a more diffuse stimulation of the brain tissue, in contrast with the more focal stimulation by the figure of eight coil, and the use of a circular coil may at least partially account for the distinct morphological features of the RRs in this sample.
- In the literature, no RRs were reported in controls using a stimulus intensity of 100% of the resting MR. The higher stimulus intensity used in this study (120% of the resting MR) may increase the presence of RRs in non-epileptic subjects. But this proposition is less likely as, with the same stimulus intensity, no RRs were elicited in the healthy controls recorded for protocol optimization (C.4.1).

The sensitivity of the RRs alone to detect epilepsy was similar in this study (63%) to that reported in the literature (60%). In this study, the RRs had a higher sensitivity to detect generalised epilepsies (69%) than focal epilepsies (54%).

In concordance with the literature, the RRs seen in epileptic patients were exclusively evoked when stimulating extratemporal structures. The same was observed for non-epileptic patients, apart from one single exception. In this case, stimulation over T5 evoked RRs, and the TMS-EEG ended up being a false positive TMS-EEG test as the patient had a final clinical diagnosis of VVS. These findings suggest that stimulating the temporal areas has a low yield for provoking RRs, and considering this fact; the TMS-EEG protocols might be modified in the future, reducing the number of stimulation areas (obviating the temporal areas) and therefore also reducing discomfort in the patient and time to perform the TMS-EEG exam.

Combined late responses (DRs and/or RRs)

The sensitivity of the TMS-EEG increases when all the TMS-EEG features, late responses (DRs and/or RRs) and IEDs are added together and considered as a whole. In this study, the combined late responses (DRs and/or RRs) were associated with the presence of epilepsy, although the sensitivity and specificity of the TMS-EEG test are reduced in this cohort in comparison to Valentin et al. work. The late responses literature is based on long-standing epilepsies. Perhaps the late responses are a less discerning biomarker of epileptogenicity at the onset of epilepsy, as is suggested for the lower specificity of the RRs and the lower sensitivity of the DRs to detect epilepsy in our sample of newly diagnosed epilepsies. Also, more than half (59%) of our epileptic sample was comprised of generalised epilepsies. In this study, the late

response, RRs and/or DRs, had a lower sensitivity to detect focal epilepsies (54%) than generalised epilepsies (69%). This may suggest that the course of the disease may influence the TMS-EEG sensitivity to detect disease, particularly in early-onset focal epilepsy.

In this study, the recording of late responses in the TMS increased the sensitivity to detect epilepsy over the EEG alone, but the specificity was compromised. The presence of additional abnormal TMS-EEG findings, namely, the IEDs, to support the classification of the TMS-EEG study as abnormal, increased the sensitivity of the TMS-EEG study to detect epilepsy. Therefore, although both the RRs and DRs increased the sensitivity to detect epilepsy over the baseline EEG when the late responses were combined with IEDs provided the highest sensitivity yield.

In this cohort, the TMS-EEG study correctly classified as epilepsy some of the patients (36%) with false-negative normal baseline EEG and a final clinical diagnosis of epilepsy, supporting a higher sensitivity of the TMS-EEG (RRs and/or DRs) to detect epilepsy than the baseline EEG alone. In most cases, the correct classification did relay in the RRs alone. This was expected as in my sample, and in keeping with the literature, the RRs were more frequently seen in the abnormal TMS-EEG than the DRs.

C.4.2.4. Summary

There has been some debate regarding the origins and meaning of the late responses, as Valentin et al. speculate that the late responses represent the scalp recording of the responses seen with intracranial electrodes after SPES. However, other authors disagree with this contention, emphasising that as TMS and electrical stimulus act over different neural targets, the TMS stimulus is likely to result in responses with a

different neurophysiological substrate than the SPES responses (Das and Nayak, 2008). The neurophysiological mechanisms underlying the generation of the late responses are not fully understood, and this subject is outside the scope of this project, although interestingly, our late responses, in particular, our RRs, differ in morphology and topographic distribution from those found in the long-standing focal epilepsy. In the visual analysis, the RRs in our sample tend to display less pronounced post-TMS-stimulus amplitude increment, resulting in a higher-than-expected interobserver variability. As a matter of fact, their morphology is more difficult to disentangle from the physiological bilateral RRs found in the controls. It may be argued that the generalised epilepsies in this sample may display singular RRs with different features than the RRs seen in focal epilepsies, but then, no characteristically distinct features in the RRs appearing in the focal epilepsies in this sample were seen.

Relying upon the late responses evoked by TMS to assign a diagnosis of epilepsy or no-epilepsy did increase the sensitivity of the TMS-EEG test in comparison to the EEG alone but in some cases added a confounding factor reducing the specificity as RRs were recorded in no-epilepsy patients. However, when considering all the abnormal features provided by the TMS-EEG, such as the presence of IEDs in addition to the late responses (RRs and/or DRs), the sensitivity significantly increased with add-on TMS-EEG (late responses+ IEDs) in comparison to the baseline EEG alone. Regrettably, the deleterious effect of the RRs in the specificity of this TMS-EEG test was not overcome. On the other hand, the DRs were highly specific, but their contribution was minimal to improving the sensitivity of the test over the baseline EEG alone. Furthermore, the TMS-EEG late responses correctly classified as epileptic a 36% of the patients with a false-negative normal baseline EEG. The subset of epileptic patients with a false-negative baseline EEG is the most pertinent for this study, but in

this subgroup, the study failed to provide a statistically significant association of the TMS evoked the late responses with the presence of epilepsy.

In view of the difficulty of establishing a reliable visual TMS-EEG feature to increase the sensitivity of the EEG without compromising the specificity, a quantitative analysis of the TMS-EEG features was performed, aiming to separate epileptic from non-epileptic subjects.

C.4.3. Quantitative analysis

The quantitative analysis of the TMS-EEG studies revealed interesting observations that, although they did not reach a level of statistical significance, showed a distinct trend between epilepsy and non-epileptic samples.

The quantitative analysis of TMS–EEG data offers the advantage of an objective assessment compared to the subjective visual interpretation of the TMS-EEG records.

One methodological approach in the literature for quantitative analyses of the TMS-evoked responses is the measure of the power of the cortical oscillations with signal processing techniques such as time-frequency wavelet decomposition. Applying this technique, following data averaged across trials will quantify the TMS time-locked oscillations (Farzan et al., 2016, Pellicciari et al., 2017).

The wavelet analysis was performed over averaged data following the average of epochs to identify the repetitive responses (RRs) which can be considered TMS evoked potentials (TEP) time locked to the TMS stimulation. For the analysis of TMS time-locked oscillations (evoked oscillatory responses-EOR), the time-frequency decomposition analysis is applied to the data averaged across trials. On the other hand, if the time-frequency decomposition is applied to single trials, all the oscillations,

including those not necessarily time-lock to the pulse (called induced oscillations), are included in the analysis (Herrmann et al., 2014), see Chapter 1, Introduction, Section C.1.3.3.6. The aim of the quantitative analyses in this study was to refine the previous visual examination of the TMS-evoked late responses to more readily identity the RRs. For this purpose, the frequency analyses of the time-locked oscillations in the time frame where RRs are expected (by definition 200 milliseconds post TMS stimulus) was selected as methodological approach. The induced oscillations no time lock to TMS were disregarded as not clearly contributing to the TEPs.

C.4.3.1. Grand average power-ratio

In this study, the power in the different frequency bands is expressed as the power ratio, the power post-stimulation divided by the power pre-stimulation for each frequency band. The power ratio offers the advantage of being a normalisation technique, reducing the measurements to the same scale and making the data comparable among subjects with interindividual variability regarding the background EEG (frequency and amplitude ranges) and anatomical variability affecting the EEG signals, e.g., cortex to skull distance, skull thickness. The brain reactivity to the TMS pulse, measured as the power ratio in particular frequency bands before and after stimulation, is suspected to be different in epilepsy and non-epileptic subjects. The contention is that the epilepsy subgroup would have different reactivity to TMS stimulation than non-epileptic subjects, and the power ratio differential (post/pre-stimulation ratio) would be a tool to separate disease from non-disease.

An anecdotal publication of quantitative EEG (qEEG) analysis of the EEG in epilepsy reported increased slow activity in the affected hemisphere of focal epilepsies (Drake et al., 1998). Other authors studying signal energy profiles over single TMS-EEG trials

showed that TMS mostly affected the brain activity within the delta-band, and this signal energy in the delta band was enhanced in the epileptic group, more so in the non-responders to AED treatment (Kimiskidis et al., 2017). Increased delta power and reduced alpha power in interictal resting EEG recording of epileptic patients have been reported by other groups (Rieg et al., 2020) (see Chapter 1, Introduction, Section C.1.2.5.2). In my analysis, the epilepsy group showed a higher power ratio in the lower frequency spectra (theta and/or delta), which is particularly noticeable upon stimulation over the posterior (O1, O2, Pz) and central (Cz) regions, suggesting an activation and/or synchronization of low-frequency bands by TMS. However, 50% of the patients in the epilepsy cohort were under AEDs treatment at the time of the TMS study. Thus, the medication's iatrogenic effect must be considered a possible confounding factor. The increased power in the delta frequency band may be partially related to the drugs' effect. Conversely, increased power in the alpha band was seen in the non-epilepsy group upon TMS-stimulation of anterior midline areas (Fz). There was an upsurge in the delta band upon stimulation of the anterior temporal areas (T3, T4) in the non-epilepsy group. Stimulation over the posterior temporal areas did not show differences between groups. These findings are of interest and more so in association with previous observations in the visual analysis where the RRs seldom appear when stimulating temporal areas in the epilepsy group. The TMS stimulation on the temporal regions might not be of paramount importance for epilepsy detection either with visual or quantitative analysis in this cohort.

C.4.3.2. Generation of a machine learning-based classification model for epilepsy prediction

The use of machine learning-based classification models has been supported by the works of other groups in the field of epilepsy and TMS (Kimiskidis et al., 2017), using

a feature selection scheme and a Bayesian classifier to separate the epileptic sample from the controls and the responders to pharmacological treatment from the non-responders. In this study, the classifier was built and validated, implementing resampling methods with iteration and stratification for variable selection. The iteration allows overcoming the small cohort size. To generate the classifier as an epilepsy prediction model, support vector machine (SVM) was used. SVM is a supervised learning model in which learning algorithms analyse the data for classification and regression analysis. The SVM training algorithm builds a model upon training samples belonging to one of two categories (epilepsy / non-epilepsy) and assigns new samples to one category or the other, making it a non-probabilistic binary linear classifier.

The performance of the TMS SVM-classifier in the TMS cohort training set has a high sensitivity, high specificity and low misclassification rate. However, the classifier was unable to separate epileptic patients from non-epileptic patients in the Sham-TMS group (i.e., using epochs of resting EEG without TMS stimulation).

When a new classifier was built with SVM upon a new variable selection in the Sham group, the new classifier performed with a similar level of accuracy in the training set in the absence of TMS activation. The new variables selected for the Sham SVM-classifier group did not overlap with the variables selected for the TMS SVM-classifier sample.

The stimulation in the midline region showed different patterns in the selected variables in the TMS sample depending on the stimulation modality A/B and the anterior to posterior location of the stimuli. When the anterior midline region (Fz) was stimulated with TMS-modality B, theta and/or gamma band variables were selected over Fp2 and C3; conversely, when the Fz region was stimulated with TMS-modality

A, no variables were selected. The pattern was reversed at the posterior (Pz) midline stimulation areas as the TMS-B modality showed only one variable; however, the TMS-modality A showed gamma, alpha and delta band variables at F3, C4 and T5 respectively. The Sham cohort showed variables at the Fz-Sham and Cz-Sham in the alpha or gamma bands with either A or B modalities. TMS did not show any variables upon stimulation over parasagittal parietal areas, while the Sham sample shows variables in the beta-gamma and delta-theta frequency ranges in these stimulation channels. These findings may suggest that the TMS selected variables were dependent on the topography and modality of the stimuli, with a distinct stimulus-dependent TMS pattern, particularly upon stimulation of the central channels. This is further supported by the apparent recording channel-stimulation area association seen with TMS but not with Sham. In the Sham, the variables appeared at the same recording channels regardless of the 'sham stimulated' area, while with TMS, the variables were identified over different recording channels as the TMS stimulation moved from one specific area to another.

After stimulation of the posterior temporal areas (T5, T6), there were no variables selected by TMS. These findings over temporal areas are in keeping with the previously noticed lack of visual findings (RRs) and the lack of power ratio differences between epilepsy/ no-epilepsy groups after TMS stimulation in posterior temporal areas. Interestingly, variables were selected upon TMS stimulation in occipital areas, in keeping with the previously reported grand average power ratio differences in the delta band seen between epilepsy/ no-epilepsy cohorts when posterior areas were stimulated with TMS.

The TMS stimulation also seemed to modify the spectral characteristics of the selected variables, which with TMS presented wider frequency ranges both in the lower (alpha-

theta) and higher (gamma) frequency ranges in contrast to the faster frequency spectrum (beta-gamma bands) of the variables selected in the Sham experiment without TMS activation.

We may conclude that the TMS stimulation modulated the spectral and topographic properties of the epilepsy-associated variables used for disease detection with machine learning linear regression algorithms.

However, the limitations of the sham group in this study have to be acknowledged. The Sham group data was obtained from averaged epochs of the baseline EEG obtained before the TMS study. The Sham data was not collected from an EEG recording performed in the same conditions as the TMS study using a sham coil. Therefore, there was no TMS sham stimulation test to verify the true stimulation effect of TMS against the placebo effect. In the Sham data for this study, the stimulation markers were randomly assigned to the baseline EEG, and therefore, TMS and Sham's protocol in this study are not entirely identical.

The prediction values of the TMS and Sham SVM classification models were assessed in an independent cohort. The Sham classifier was a poor prediction model as it had a low sensitivity to predict epilepsy, below 70%. One possible explanation for the high performance of the Sham SVM classifier in the validation set but the poor prediction of disease status in the independent cohort is that the Sham-SVM classifier may fit the training data too tightly. Overfitting may occur in highly complex models with an excessively high ratio of the number of parameters versus the number of observations. In complex models may be difficult to determine which variables are noise to be ignored and which variables are truly associated with the disease (Domingos, 2012).

In contrast to Sham, the TMS SVM-classifier was a better prediction model showing a higher sensitivity than Sham to predict epilepsy.

The sensitivity of the TMS SVM-classifier was 83%, but the specificity was low (30%).

The literature on machine learning for epilepsy detection shows previous studies implementing the use of machine learning models over EEG features. Kimiskidis et al. study showed paired-pulse TMS-EEG epilepsy-associated features using a Bayesian classifier to predict/ separate epileptic patients with GGE from controls with high sensitivity and specificity (86% and 82%, respectively). Another study applied SVM algorithms before and after medication and achieved an 86 % sensitivity and 77% specificity for responsiveness to treatment classification (Ouyang et al., 2018). A breakthrough pilot study aiming to detect epilepsy disease applying random forest algorithms for the selection of epilepsy-associated spectral analysis features (fine band graded frequencies) extracted from seizure-free resting EEG recordings obtained a balanced accuracy of 75.6 % for epilepsy prediction on EEG independent data new to the algorithm (Buettner et al., 2019, Rieg et al., 2020). In the present study, the sensitivity and specificity of the TMS SVM classifier are lower than in Rieg et al. study. Some confounding variables in the prediction dataset may account for this lower sensitivity and specificity for epilepsy prediction in our classifier in comparison with the literature. Some of these confounding variables may be clinical (different proportion of epilepsy subcategories between training and prediction datasets) or relate to variability in TMS-EEG protocol (see material and methods' chapter, section C.2.3.5., classifier prediction of epilepsy in an independent cohort). However, the sensitivity of the TMS SVM classifier epilepsy prediction model was still above 70%, which is still considered in the literature as a valuable contribution to epilepsy detection. Increasing the number of patients in the training set may improve the

model’s accuracy. This opens promising avenues for future work to improve the accuracy of the classifier by increasing the number of patients in the training and validation sets and by providing a more representative sample of the various epilepsy subtypes for analysis.

CHAPTER 5. CONCLUSION

The TMS-EEG study is a generally well-tolerated test that aids in the earlier detection of epilepsy. The results of this study suggest that the TMS evoked EEG late responses are associated with the presence of epilepsy and increase the sensitivity of the baseline EEG for epilepsy. The DRs are a TMS-EEG feature highly specific and strongly associated with epilepsy but difficult to elicit and did not have a significant impact on increasing the sensitivity of the TMS-EEG. The other type of TMS evoked EEG responses, the RRs, increased the sensitivity of the test to detect epilepsy, but the RRs had a detrimental effect on the specificity as RRs were recorded in non-epileptic patients. In the subset of patients with a normal baseline EEG, the late responses correctly classified as epileptic a 36% of the patients with a final clinical diagnosis of epilepsy, supporting a higher sensitivity of the TMS-EEG to detect epilepsy than the baseline EEG alone.

The grand average power-ratio power ratio differences between epilepsy and no-epilepsy cohorts were not statistically significant, but there was a tendency for the epilepsy group to show a higher power ratio in the theta and delta bands, particularly when the TMS stimulation was applied in the posterior and central midline brain regions. Conversely, the non-epilepsy group tended to display a higher power in the alpha band upon TMS stimulation of the anterior brain regions. Stimulation over the posterior temporal areas did not show differential trends between groups.

The TMS stimulation seemed to modify the spectral characteristics of the epilepsy-associated variables selected for machine learning-based classification. These variables had lower frequency ranges than the variables selected when TMS activation was not present. The topographic pattern of these selected variables in the recording channels appears to vary depending on the area and modality of TMS stimulation.

C.5. Conclusion

The TMS SVM-classifier was a superior epilepsy prediction model than the Sham, suggesting that the machine learning SVM model may offer additional value for disease prediction in TMS-EEG datasets.

Further improvement in the accuracy and prediction power of the classifier with larger and more curated training datasets and external validation of the TMS SVM classification model are exciting avenues for future work.

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Appendix

Table Patient's clinical details						
P	**Sex**	**Age**	**Preliminary Clinical Findings from the First Seizure Clinic**	**Sz**	**Diagnosis**	**FCC**
1	F	48	An episode of blackout leading to a minor road traffic accident and persistent memory gap after the incident, unlikely to be epileptic seizures, thought to be stress-related and non-epileptic in nature Neuropsychiatric comorbidities: migraines treated with Eleptriptan		PNES	NE
2	F	32	She was referred for a convulsion during sleep, shaking and struggling to breathe, prompt recovery, no incontinence or tongue biting. Three similar episodes between 6 and 8 years ago were triggered by stress		PNES	NE
3	F	53	Three episodes of LOC and generalised convulsion and urinary incontinence, on one occasion, followed by vomiting and prompt recovery. The last event occurred 24 hours prior to the TMS-EEG-sleep deprived study		PNES	NE
4	F	17	He was referred by A&E after a generalised convulsion. Four generalised episodes of falls, shakiness, stiffness and generalised convulsion and preceded by blurred vision. These events were clinically thought to be non-epileptic in nature.		PNES	NE
5	F	35	Episodes characterised by 5 to 15 min. of generalised shaking not preceded by a warning and followed by a confusional state. Hx of epilepsy since the age of 18, diagnosed and managed abroad, four to five episodes a year of diffuse convulsions treated with a combination of Carbamazepine and Levetiracetam. The last attack occurred 3-4 months prior to the TMS-EEG-sleep deprived study	G	GGE/ GTCS only	E

6	F	31	Episode characterise by LOC preceded by a prodromal of mood change or irritability and followed by fall and shakiness for about five minutes Hx: epilepsy since the age of 13, with one seizure every six months, mixed of genuine and stress-related events, treated with lamotrigine. Neuropsychiatric comorbidities: sustained hypoxic brain injury in 2007 leading to short-term memory loss	G	GE	E
7	F	51	4-5 generalised convulsions with urine incontinence and tongue biting in the last 12-18 months, classified as GTCS and frequent episodes of blank spells and unresponsiveness thought to be absences, currently treated with levetiracetam Hx of JAE was diagnosed at the age of 11	G	IGE/ JAE	E
8	F	22	Frequent upper body and arms' myoclonic jerks, monthly episodes of generalised convulsions characterised by LOC, body rigidity, eyes rolling back and clicking mouth sounds lasting approximately 2 minutes and followed by long periods of tiredness/ sleepiness. The last GTCS occurred three days prior to the sleep deprived-EEG-TMS study. Currently, she is treated with levetiracetam. Hx of JME diagnosed at the age of 18	G	JME	E
9	M	17	Episodes of "blank spell" typically lasting between 5-10 seconds and occasionally more prolonged vacant episodes and unresponsive lasting up to 5 minutes. Strong family history of idiopathic generalised with absences Other neuropsychiatric comorbidities: dyslexia and dyspraxia		PNES	NE
10	M	30	Referred by A&E following six generalised convulsions thought to be acute symptomatic seizures in the context of sleep deprivation and illicit drug misuse of methamphetamines and gamma-butyrolactone. There is a strong family history of epilepsy. Neuropsychiatric comorbidities: occasional panic attacks	AS	AS	NE
11	M	59	A single episode of loss of consciousness happened in the context of severe back pain. The recovery was almost instant, and the event was not accompanied by convulsions. There was no strong clinical evidence for epilepsy, and the event was clinically thought to be a neurally mediated syncopal event		VVS	NE

12	F	39	A single episode during sleep of reduced sensation and mild weakness of the right side of the body lasting for the whole of the next day. Previous Hx of episodes of LOC at of 12-13 years of age investigated with EEG, which showed nonspecific findings		PNES	NE
13	F	32	An episode of loss of consciousness occurred after excessive consumption of alcohol. The episode was accompanied by incontinence and possible confusion after the event. A previous Hx of a possible first seizure at the age of 18 years and a second event at the age of 22, investigated abroad with an EEG which showed photosensitivity. Both events occurred in nightclubs, probably under strobe lights.		PS/ (mild)	E
14	F	34	Episodes of LOC, head-turning to the right and right-side shakiness followed by 1-2 minutes of generalised convulsion followed by exhaustion and headache. Previous Hx of JAE since the age of 15 and currently treated with phenobarbitone and Levetiracetam	G	GGE/ JAE	E
15	M	32	Two recent events of generalised convulsions were related to disturbances in the sleep-wake cycle and stress. Hx: ELMA since the age of 8, currently under treatment with Valproate, Levetiracetam and Lamotrigine.	G	ELMA	E
16	M	23	Episode of secondary generalised seizure Hx started at the age of 17 with episodes characterised by migraine, blurred vision or sudden flashing lights followed by LOC and body shaking for 2-5 minutes. MRI showed right mesial temporal sclerosis. Currently under treatment with carbamazepine and clobazam	F	R TLE	E
17	M	30	Generalised convulsions associated with tongue biting preceded by a feeling of dizziness and light-headedness, feeling hot and sweaty. These features may raise further questioning of neurally mediated syncopal events. Previous Hx IGE with GTCS under control with Valproate	G	GGE/ GTCS only	E

18	M	27	A single episode of loss of consciousness and collapsing on the floor was associated with some convulsions and followed by a fairly quick recovery. The event was preceded by hot and cold flashes early on the day, and a sensation of feeling “strange”, and these features clinically raise the possibility of vasovagal syncope		VVS	NE
19	F	39	She was referred for possible seizure recurrence after an episode of confusion and tremor preceded by a stomach’s “butterflies” sensation. Profuse perspiration, weakness and vomit accompanied the event, which seems more likely to be vasovagal-neurally mediated syncope in nature. Hx of epilepsy diagnosed abroad at the age of 15, seizure-free since the age of 31 and 5 years off carbamazepine		VVS	NE
20	F	18	An episode of LOC, with no prodromic symptoms recalled by the patient and relative, described unresponsiveness and convulsion for 5-10 minutes. There was tongue biting but no incontinence. Previous Hx: recently diagnosed with vasovagal syncope, a previous EEG suggestive of right frontopolar epileptogenicity.	F	R FLE	E
21	M	17	Episodes of unresponsiveness were described as blank spells lasting up to 5 minutes which clinical features were against absences or epileptic seizures and thought to be probably non-epileptic events. Hx of possible absences at the age of 8, which were never confirmed, currently taking Valproate		PNES	NE
22	M	32	He was referred by A&E after a first generalised convulsive seizure at work, without warning, thought to be probably late-onset post-traumatic focal epilepsy. Previous Hx of serious head injury at the age of 4 years. Imagining studies showed left frontal gliosis	F	L FLE	E
23	F	43	New episodes of “déjà vu” started a year ago and are clinically suggestive of focal seizures. Hx: epilepsy since the age of 20 years treated with Valproate and tendency to vasovagal syncope. MRI: hippocampal asymmetry with mildly increased signal in the right dentate gyrus	F	R TLE	E

24	F	33	Prolonged events of disturbed awareness raise the question of absence status epilepticus. Brief (a few seconds) staring episodes occur diary and often in clusters. Free of GTCS for a year. Hx of absence seizures, staring and eyelid blinking events since the age of 6-7, diagnosed with IGE (ELMA) at the age of 11, and GTCS started at the age of 17 with an average of one GTCS per year. Treated with Valproate	G	GGE/ ELMA	E
25	F	70	An episode of convulsion while recovering from epidural anaesthesia was clinically thought to be an acute symptomatic event rather than a recurrence of epilepsy. Hx of episodes of loss of consciousness at the age of 50 were diagnosed as epilepsy and treated with carbamazepine which rendered the patient seizure-free until the current event. A 200-mgr. dose of carbamazepine in the evening is still maintained.	AS	AS	NE
26	F	22	Referred from A&E for episodes of generalised convulsions accompanied by urine incontinence and lip biting, lasting less than a minute, not preceded by any warning and followed by prompt recovery. These were clinically thought to be GTCS. Hx of infrequent episodes of LOC and generalised convulsions started at the age of 12, occurring approximately every six months. Head injury at the age of 13 may raise the questions of cryptogenic generalised epilepsy. Currently, she is treated with carbamazepine.	G	GGE/ GTCS only	E
27	M	32	The first episode of loss of consciousness was not preceded by any warning. The witnesses described the patient making a loud noise and shaking for approximately 2-3 minutes. The patient regained consciousness 20 minutes later but remained tired and confused for a while after.	G	GGE/ PA	E
28	F	34	She was referred by A&E for episodes of loss of consciousness. The events are stereotypical, consisting of a sensation of pressure on the chest and feeling out of breath followed by falling into the ground and presenting regular and slow frequency chest movements. The clinical features are not in keeping with epilepsy, the stress is a precipitant, and it appears that psychogenic and vasovagal mechanisms coexist.		VVS/ PNES	NE
29	M	25	A single episode of LOC and generalised convulsion preceded by 1-2 minutes rising epigastric sensation. The witnesses described a scream and violent convulsions for approximately 5 minutes. There was no tongue biting or incontinence. The patient felt tired and confused after the event. The episode was clinically suggestive of probable focal TLE in spite of the normal EEG and MRI investigations.	F	TLE	E

30	F	20	Mostly nocturnal episodes are characterised by dizziness, light-headedness, blurred vision and a feeling of becoming warm and sweaty. Clinically, there is a strong vasovagal element, questioning the presence of vasovagal syncope		VVS	NE
31	F	29	Nocturnal episodes during wakefulness or early drowsiness of shaking lasting 5-10 minutes. During the episodes, the consciousness is impaired, although the patient may be able to appreciate the shaking. After the event, she feels tired and excessively sweaty. There is no strong suggestion of epileptic nature.		PNES	NE
32	F	28	Two episodes of loss of consciousness presented with ringing and buzzing in the ear and then blurring or loss of vision followed by LOC, which quickly recovered in less than a minute. The clinical picture is thought to be consistent with vasovagal activity. Neuropsychiatric comorbidities: mild depression and obsessive-compulsive disorder, currently treated with Carbamazepine 200 mgrs.		VVS	NE
33	M	53	An episode of LOC preceded by dizziness and blurred vision and clinically questioning the presence of a neurally mediated syncope		VVS	NE
34	M	26	Referred by A&E following an episode of loss of consciousness and convulsions, not preceded by a warning. The event cannot be fully characterised, but when the witnesses arrived at the scene, they saw the patient “fitting and shaking”, and he remained very confused for 40-50 minutes after the event. There was no tongue biting or urine incontinence	F	L FLE	E
35	F	39	Two types of events were reported: dystonic events and fleeting episodes of loss of consciousness. The latter was thought to probably be long-standing PNES. Unfortunately, the patient was lost to follow up; therefore, the nature of the events could not be fully characterised, and the data were excluded from the study.			*

36	M	18	Episodes of “blackout” and LOC are clinically suggestive of vasovagal syncope following an assault and minor head trauma. The episodes occurred while standing or walking and were preceded by a feeling of dizziness, light headiness, progressive darkening of the vision and narrowing of the visual fields. The patient then would feel weak fall onto his knees, and this would be his last recollection. There is no tongue biting or urine incontinence, and there is a prompt recovery after 2-3 minutes.		VVS	NE
37	F	90	Frequent episodes of brief vacant spells, lasting 10-30 seconds and occurring every 15-20 minutes. The events are thought to be non-epileptic blank spells. Currently, she is treated with Valproate. Other neuropsychiatric comorbidities: advanced dementia Alzheimer’s disease; therefore, the patient was not included in the study.			*
38	M	38	An episode of blackout associated with tongue biting and incontinence occurred in the context of stress and lack of sleep. The event was preceded by “shivering” and feeling “jittery “and followed by a prompt recovery.		VVS	NE
39	M	32	An episode of confusion and disorientation happened in the context of stress. Other neuropsychiatric comorbidities: depression		PNES	NE
40	F	29	An episode of collapse while standing, not preceded by any clear warning apart from a split-second feeling of light-headedness. There was no tongue biting or urine incontinence. Prompt recovery in 1-2 minutes. Querying photosensitivity as the event occurred at a performance with loud music and strong strobe lights		VVS	NE
41	M	22	An episode of loss of consciousness associated with convulsions occurred without previous warming while he was standing. There was no tongue biting or urinary incontinence, and the recovery was quick. Hx of muscle twitching and possible brief blank spells since childhood, no investigated at the time. Head injury at the age of 8-9		VVS	NE

42	M	27	A recent episode of left arm shaking while in clear consciousness during 1 minute and then followed by a 5-10 minutes blackout. No tongue biting or urinary incontinence. Hx of an episode of shaking, the first at the age of 13, two more events at the age of 23, sometimes accompanied by loss of consciousness; in other occasions, milder episodes of diffuse shaking could be aborted by concentration. There is no clear evidence for epilepsy, and the episodes appear to be stress-related.		PNES	NE
43	M	44	One single nocturnal episode was characterised by stiffness and jerking of the right arm, right leg and face. The patient was conscious during the event, which lasted approximately 5-10 minutes and was followed by a prolonged period of confusion. The clinical description favours left-sided focal epileptogenesis. Hx: he suffered two very severe head injuries and alcohol abuse more than ten years ago.	F	R FLE + L TLE (BF)	E
44	M	57	A single unexplained first episode of loss of consciousness occurred while standing, without any warning. There was stiffness and togue biting but no convulsions or urinary incontinence. Previous Hx on RTA in 1999, prosthetic right eye			NE
45	F	28	Recently a single generalised convulsion related to sleep deprivation and alcohol. Hx of well-controlled juvenile myoclonic epilepsy since the age of 11, currently treated with levetiracetam (125 mgr. once a day).	G	JME	E
46	F	32	An episode of generalised convulsion occurred while on holiday abroad. There was no warning, and it is not clear if she was confused after recovery. There was no tongue biting or urinary incontinence. There is Hx on a head injury in a road traffic accident at the age of 17. Currently treated with carbamazepine (400 mgr. twice a day).	F	R FT	E
47	M	21	A single episode of loss of consciousness occurred in a train station. He recovered at the scene 20 minutes later. There was tongue biting but no urinary incontinence. Clinically uncertain, although possible first seizure.			NE

48	F	32	The episodes consist of deja vu experience, associated with some disturbance of awareness. The symptoms would last for less than a minute, and the recovery is quick, but the patient may feel tired and groggy after a cluster. Over the last year, the frequency of the episodes has been reduced to once every six months, and there are no clusters. These events may turn out to be focal experiential ictal symptoms. Hx; diagnosed with temporal lobe epilepsy since 2010 due to deja vu episodes which occurred monthly, up to 8 times on the same day, the treatment with topiramate and lamotrigine was discontinued a few months ago.	F	R TLE	E
49	M	21	He was admitted to the hospital following two generalised convulsions at home and a third seizure at A&E. he was found shaking in bed with his eyes open. This was followed by a period of agitation and confusion. According to the patient, he has been having clusters of seizures intermittently for the past six months. Treated with sodium valproate.	F	L focal / L H	E
50	M	23	A first generalised convulsion occurred in the context of methylenedioxymethamphetamine (NMDA) consumption.	AS	AS	NE
51	F	37	An episode of LOC at work associated with generalised convulsions lasting 5-7 minutes. There were no warnings. Since this event, the patient experienced two further episodes at home, where the face became contorted, followed by a brief LOC and feeling sleepy afterwards. Previous Hx ten years ago of a brief episode characterised by light-headedness as she stood up, followed by LOC, shaking of limbs, urinary incontinence and tongue biting. 3-4 years ago, there was a brief episode of LOC with vasovagal clinical features in the context of severe pain.	G	GGE/ GTCS only	E
52	M	49	An episode characterised by LOC, limb twitching and tongue biting. Hx of vasovagal symptoms, dizziness and unsteadiness during postural changes and light handedness in the morning.		VVS	NE
53	F	18	She was referred by A&E following an episode of LOC. Prior to the event, she felt woozy, lightheaded and hot; her vision became blurry and then she collapsed to the ground. There was no tongue biting or incontinence, and the recovery was quick. She experienced similar symptoms in the past without losing consciousness. She reports muscle twitching when she drifts off to sleep but also during the day.		VVS	NE

54	F	43	Two unexplained episodes of LOC associated with convulsions occurred without any warning. There was tongue biting and urinary incontinence. The events were clinically thought to reflect a lower threshold for epileptic seizures despite the normal routine EEG	NC	GE	E
55	F	53	Two events of LOC and shakiness were thought to be non-epileptic in nature. The first episode was characterised by an out of body experience while sitting on the couch watching TV, followed by shaking of the limbs. She did not lose awareness until she stood up, fell to the floor and lost consciousness for a couple of minutes. After she woke up, she felt confused for an hour. No warning preceded the events. Hx of epilepsy between the age of 3 – 12 and seizure-free since then. Neuropsychiatric comorbidities: she suffers from depression, currently treated with Sertraline.		PNES	NE
56	M	49	One episode of LOC while sitting in front of his desk. He complained of tunnel vision preceding the event. He was noted to jerk for about a minute. The was no tongue biting or incontinence. Hx: there was a previous episode of blackout without warning. He was unconscious for at least 10 minutes. This happened after a substantial amount of alcohol consumption.		VVS	NE
57	M	31	He was referred by A&E following an episode of LOC associated with convulsions. He felt hot and sweaty prior to the event, and he recovered 5-10 minutes later. He had a similar episode 2 years ago and a previous Hx of vacant spells in his late teen early twenties, which apparently still persist. Other neuropsychiatric comorbidities: neuropathic pain treated with various pain killers (pregabalin), headache, and anxiety disorder.		VVS	NE
58	M	20	Two generalised convulsions in the space of one hour, following a night of poor sleep and excessive alcohol consumption. These events were clinically thought to be tonic-clonic seizures. The first episode was witnessed at work where he was found convulsing, with his hand rhythmically beating on the desk; he slumped off his chair and fell to the floor. Afterwards, he was not properly responding to his colleagues. The second event occurred in the ambulance. There was tongue biting but no urinary incontinence.	G	GGE/ GTCS only (mild)	E
59	F	56	An episode of LOC while entering a train station. She suddenly felt dizzy, and her last recollection was recovering one hour later. There was no urinary incontinence or tongue biting. A week later, she experienced intense dizziness again, but she did not space out, and her memory for the event was intact.		VVS	NE

60	F	22	Episodes of LOC preceded by a brief “fluttering sensation” in the whole body; then she loses awareness and becomes unresponsive and shaky for around 1 minute. There is no incontinence, but she has bitten her lip or the inside of her mouth. There is confusion following the events. Hx of similar events, while she was abroad, was thought to be epileptic, and she is currently treated with levetiracetam.	G	GGE/ GTCS only	E
61	M	42	One episode of convulsive movements occurred after going to bed at 2.00 a.m. The event was characterised by initial “mumbling” followed by convulsions, unresponsiveness for 1-2 minutes and confusion for around 5 minutes. There was no tongue biting or urinary incontinence. The episode was thought to be a nocturnal generalised tonic-clonic seizure. Currently treated with levetiracetam.	F	R FLE	E
62	F	36	An episode of tingling sensation in her head followed by LOC when entering a shop with bright light. This raises the question of possible photosensitivity Hx of an event of LOC at the age of 11 years, while she was sitting in front of a computer at school.			NE

P, patient; **FCC**, final clinical classification; **E**, Epilepsy; **NE**, Non-epilepsy; **Sz**, seizure type; **G**, generalise seizure; **F**, focal seizure: **VVS**, vasovagal syncope, **PNES**, psychogenic non-epileptic seizure; **GGE**, genetic generalised epilepsy: **GTCS**, generalised tonic-clonic seizures; **JAE**, juvenile absence epilepsy; **JME**, juvenile myoclonic epilepsy; **ELMA**, eyelid myoclonia with absences; **PA**, phantom absences; **TLE**, temporal lobe epilepsy, **FLE**, frontal lobe epilepsy; **FT**, frontotemporal; PS, photosensitive epilepsy; **BF**, bifocal; **AS**, acute symptomatic seizure; **R**, right; **L**, left; **E(NC)**, epilepsy not possible to classify; **Hx**, clinical history; **LOC**, loss of consciousness; **(*)**, excluded from the study

Appendix 1. Summary table of the most relevant clinical data of the 62 participants recruited for the study from the first seizure clinic. In the table, the seizure type (Sz) refers to the event for which the patient was referred to the first seizure clinic. In some instances, the patient suffered from epilepsy in the past, and they returned to the clinic after being asymptomatic for a lapse of several years. Upon further scrutiny in the first seizure clinic, other patients mentioned previous events before the one for which they were referred, which were not fully investigated at the time. In some cases, there was an additional episode in the interval between the referral to the first seizure clinic and the subsequent appointment for the clinic and /or the sleep-deprived TMS-EEG.