Aus dem

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Altered brain state dynamics in generalized epilepsy

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List of abbreviations

AC	Average condition
AR	Appearance rate
AUD	the Auditory Network
BH	Benjamini–Hochberg

BOLD	Blood oxygenation level–dependent
CAE	Childhood absence epilepsy
DEE	Developmental epileptic encephalopathy
DMN	the Default Mode Network
DT	Dwell time
DRE	Drug-resistant epilepsy
EEG	Electroencephalography
FC	Functional connectivity
FED	Focal epileptiform discharge
FDR	False discovery rate
fMRI	Functional magnetic resonance imaging
FO	Fractional occupancy
FPN	the Frontoparietal Network
GED	Generalized epileptiform discharge
GGE	Genetic generalized epilepsy
HIC	High-income countries
IC	Independent condition
ILAE	the International League Against Epilepsy
IPC	Inferior parietal cortex
JAE	Juvenile absence epilepsy
JME	Juvenile myoclonic epilepsy
LCMV	Linear constrained minimum variance
LFPs	Local field potentials
LMIC	Low- and middle-income countries
MAD	Median absolute deviation
MEG	Magnetoencephalography
MRI	Magnetic resonance imaging
RBF	Radial basis function
RSFC	Resting-state functional connectivity
SC	Structural connectivity
SNR	Signal-to-noise ratio
SQUID	Superconducting Quantum Interference Device
STD	Standard deviation
SVM	Support vector machine

TBI	Traumatic brain injury
TLE	Temporal lobe epilepsy
TP	Transition possibility matrix
VIS	the Visual Network

1 Introduction

The human brain is a highly interconnected network, with different brain regions collaborating to achieve various functions. Sequential activation of different brain regions over time forms a range of different functional brain states. These brain state dynamics, the changes in brain states over time, underlie the execution of different functional and cognitive processes. Traditionally, brain networks have been studied in a static manner, focusing on functional connectivity and largely ignoring the adaptive nature of the brain. In this thesis, I propose a dynamic network approach, analyzing the co-activation of different brain regions over time - referred to as brain states - using magnetoencephalography (MEG) data with high temporal resolution to better characterize brain dynamics.

The reconfiguration of neural networks during information processing might be disrupted or impaired under pathological conditions, with epilepsy being one such example. Epilepsy is recognized as a network disorder with proven altered functional connectivity. We hypothesize that the dynamics of brain states in epileptic patients are also altered and may better describe the mechanisms of epileptic pathogenesis. By applying machine learning algorithms, I identify and compare these brain state dynamics between epileptic patients and healthy controls to reveal altered brain dynamics in epileptic patients.

1.1 Brain networks

The human brain is a dynamic system continuously processing large amounts of input and output information to enable perception, cognition and behavior (Cornblath et al., 2020, Fornito et al., 2016). Considered as the most complex human organ, it comprises approximately 100 billion neurons connected by around 100 trillion synapses in a multi-scale anatomical organization. Communication between neurons from different brain regions generates complex inherent interactions within the brain, known as brain networks (Fornito

et al., 2016). Brain networks are categorized into two general types: Structural (anatomical) networks and Functional networks, based on different types of connectivity (Betzel et al., 2017). Structural connectivity (SC) of a brain network describes the patterns and the integrity of white matter connections between neural populations, whereas functional connectivity (FC) of a brain network describes patterns and strength of temporal associations of activation patterns across remote brain regions (Litwinczuk et al., 2022). For example, in MRI studies, white matter fiber tracts reconstructed with tractography algorithms are considered as Structural connectivity (SC). Functional connectivity (FC) is defined as the statistical relationship (Fisher-transformed correlation coefficient or a coherence measure) of activities between brain regions over time (Betzel et al., 2017). The synchronized fluctuations of functional connectivity are thought to reveal the brain's intrinsic network organization (Mill et al., 2020). Structural connectivity is considered stable within a certain period of time (Sporns, 2013) and is there independently of its involvement in a certain task. While Functional connectivity between two areas might exist even without the existence of anatomical connection.

In this study, we focus on functional brain networks. Over the past two decades, resting-state studies have facilitated the understanding of human brain function via resting-state functional connectivity (RSFC), that is, the statistical estimation of ongoing brain signals from spatially separate brain regions corresponding to known functional systems (Laumann et al., 2017, Brookes et al., 2011). The most used method for RSFC is the sliding window technique, in which correlations of activity between brain regions over time are estimated at continuous time points within a fixed duration (approximately 100 seconds). A matrix of pairwise correlations across brain regions is calculated to construct large-scale functional networks (Zalesky et al., 2014, Allen et al., 2014). In addition, task-based studies have been conducted to explore the functioning of brain networks by applying cognitive, sensory, or motor tasks to observe

changes in brain signals during task performance relative to rest or control periods (O'Neill et al., 2017, Seitzman et al., 2019a). Numerous brain network studies (Yeo et al., 2011, Damoiseaux et al., 2006) have identified a range of brain networks, and we list some typical functional networks as follows:

The Default Mode Network (DMN) consists of regions in the anterior pre-frontal cortex, posterior cingulate cortex, retrosplenial cortex, medial and lateral parietal. It is commonly activated significantly at rest and relatively deactivated during tasks requiring attention (Rosazza et al., 2011).

The Visual Network (VIS) consists of the striate cortex (V1, Brodmann area 17) and some extra-striate areas in the occipital lobe. Like the Somato-Motor Network (SMN), these regions are of great concern to neurosurgeons seeking to avoid any perceptual or motor deficits. (Rosazza et al., 2011).

The Auditory Network (AUD) involves regions such as the superior temporal gyrus, the Heschel's gyrus, the insula, and the postcentral gyrus. AUD is related to language perception and to reading (Rosazza et al., 2011, Seitzman et al., 2019b).

The Frontoparietal Network (FPN) is a set of brain areas in dorsolateral prefrontal cortex, the inferior parietal lobule, the middle of the middle temporal gyrus, and a dorsomedial prefrontal region anterior and superior to anterior cingulate cortex. The FPN, also known as the prefrontal control network, is the mediator between the other brain networks and responsible for top-down goal-directed control processes (Scheffer et al., 2017).

Brain networks play an important role in explaining the brain's functioning in physiological and pathological conditions. For example: growing evidence shows that altered brain networks such as the default network, frontoparietal network, dorsal attentional network and visual network are involved in impaired cognitive functioning in patients with Parkinson's disease (Filippi et al., 2019). He et al. demonstrated that language network as a specialized and integrated

system, whose transient dynamics are disrupted in patients with temporal lobe epilepsy (He et al., 2018).

Functional connectivity builds stationary functional networks that are stable within a time window. However, evidence from multiple modalities suggests that functional state patterns (functional networks) are dynamic over time (Bressler et al., 2001, Deco et al., 2011, Rabinovich et al., 2006). Neuroscientists begun to pay attention to the organizing principles of the large-scale network dynamics that characterize this time-varying and intrinsically coupled activity across spatially distributed and functionally differentiated cortical areas (Peng et al., 2023). These transient network configurations and their manifestation of spatial-temporal dynamics have been reported at both rest (Allen et al., 2014) and task (Du et al., 2018). The examination of single-volume MR images (rather than averaging over a windowed period) have allowed for the decomposition of blood oxygenation level–dependent (BOLD) signals into their underlying time-varying brain coactivated patterns, which sum to form large-scale intrinsic networks (Cornblath et al., 2020, Peng et al., 2023). These repetitively activated large-scale networks are called "brain states". Such a co-activation-based analysis method allows to detect transient functional network interactions occurring on a smaller time scale and therefore to better estimate their temporal dependence. Notably, this approach is powerful for investigating the brain network (brain state) dynamics that evolve across spatially distinct and functionally differentiated brain regions reflecting neural representations of processing different information (Peng et al., 2023, Cornblath et al., 2020). These brain state dynamics can be quantified using some parameters (Cornblath et al., 2020, Baker et al., 2014) such as: **Fractional occupancy (FO)** represents the probability of a brain state α occurring, indicating the proportion of time the brain spends in that specific state. (Farinha et al., 2022). **Dwell time (DT)** the dwell time (mean duration) of a brain state represents the mean consecutive epochs spent in that state throughout a measurement (Farinha et

al., 2022). **Appearance rate (AR)** the appearance rate (mean duration) of a brain state represents frequency of appearance per unit of time of that state throughout a measurement (Olafson et al., 2022). **Transition possibility matrix (TP)** the probability of brain state α transition to brain state β from time t to time t+ 1(Farinha et al., 2022). It has been shown that alterations in these brain state dynamic parameters show potential as physiological markers for disease diagnosis and prognostic assessment, e.g., increased DT and FO of the frontoparietal and stomato-motor networks correlate with a favorable prognosis for stroke recovery (Olafson et al., 2022, Tang et al., 2024).

Summary: Brain networks are associated with different brain functions. These networks are independent and are referred to as "brain states" when they are activated. These brain states dynamically evolve over time according to certain rules. Altered brain state dynamics may be characteristic of certain diseases. While coherence (functional connectivity) based network analysis studies the stationary flow of information from one brain region to another, the approach here studies dynamic changes in brain states, i.e., statistical sequences of brain states. Presumably, this approach reflects the processing of information better than any stationary description of brain activation.

1.2 Overview of Magnetoencephalography (MEG)

Neuroimaging technologies have revolutionized our understanding of brain by providing critical insights into the structure and function of the human brain. These techniques play a crucial role in neuroscience research, allowing scientists to study brain dynamics in both healthy individuals and patients with neurological disorders. Among the most widely used neuroimaging methods are functional magnetic resonance imaging (fMRI), electroencephalography (EEG), and magnetoencephalography (MEG). Each of these techniques offers a unique perspective on brain activity.

1.2.1 Magnetoencephalography (MEG)

Magnetoencephalography (MEG) quantifies brain activity by non-invasively recording the magnetic fields generated by the electrical activity of neuronal populations. Benefitting from the Superconducting Quantum Interference Device (SQUID), a remarkably sensitive magnetometer that enables MEG to measure extremely small magnetic field fluctuations (~ tens of fT).

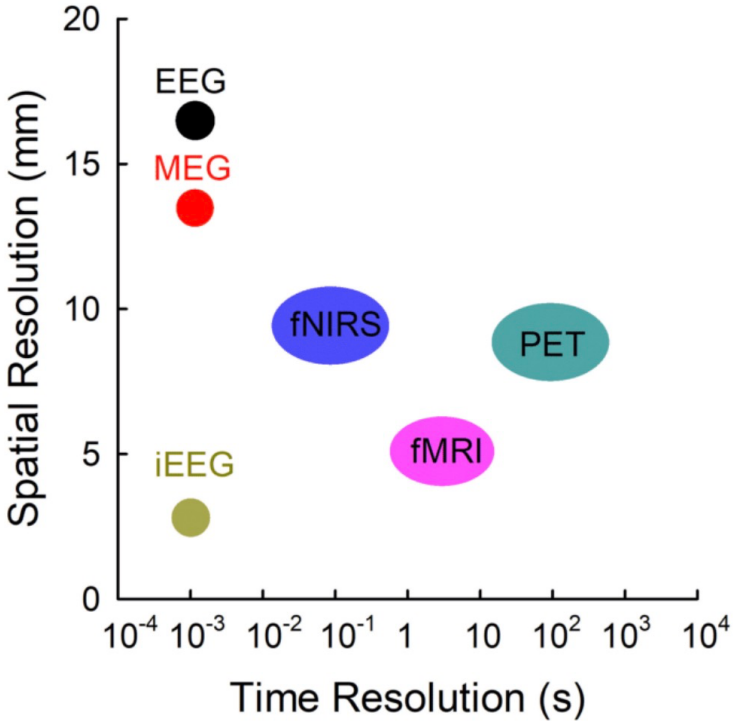

Fig. 1. Comparison among different functional imaging modalities(Xu et al., 2021).

1.2.2 MEG vs fMRI

Blood oxygen level-dependent (BOLD) functional magnetic resonance imaging (fMRI) measures changes in deoxyhemoglobin and oxyhemoglobin concentration indirectly quantifying neurometabolism to reflect the cerebral functional status. However, the relatively slow speed of hemodynamic changes in response to neuronal activity inherently constrains the temporal resolution to a timescale of several hundred milliseconds (Glover, 2011) **(Fig.1)**. In contrast, magnetoencephalography (MEG) provides direct measurements of magnetic field changes induced by intracellular current flow, offering a millisecond-level temporal resolution of the neuronal electrical currents. MEG permits to capture the neural dynamics from a few milliseconds to hundreds of milliseconds. Different from fMRI, which generates gradient coil noise, MEG is a silent recording technique providing a more relaxed and comfortable environment for participants during data acquisition.

1.2.3 MEG vs EEG

EEG and MEG, measure local field potentials (LFPs) and local magnetic fields (LMFs) generated by postsynaptic potentials, respectively. EEG is sensitive to extracellular current components, while MEG is sensitive to intracellular current components (Lopes da Silva, 2013). Since anatomical structures (cerebrospinal fluid, skull, and skin) have a stronger distortion effect on EEG than MEG, MEG provides higher spatial resolution than EEG. On the contrary, magnetic field fluctuations decrease rapidly with distance, which limits the sensitivity of the MEG to deep sources. EEG has been widely adopted due to its affordability and broad accessibility, whereas MEG, due to its high purchasing and maintenance costs, is only affordable for a few neuroscience centers. Fortunately, the latest cost-effective MEG solutions herald the possibility of widespread installation.

1.2.4 MEG application

MEG is applied in neuroscience for the diagnosis of neurological disease diagnosis, for studying cognition, and for the identification of clinical biomarkers. The initial clinical application of MEG was for the pre-operative localization of the epileptogenic zone localization. MEG has been increasingly used in clinically orientated studies such as stroke (Laaksonen et al., 2013), autism (Bailey et al., 2005), schizophrenia (Dima et al., 2012), Alzheimer's disease (de Haan et al., 2012), depression (Laaksonen et al., 2013), attention deficit hyperactivity disorder (Dockstader et al., 2009). Interestingly, a large number of MEG studies focused on brain rhythms and spectral features which contribute to our knowledge of underlying neural mechanisms for a set of cognitive tasks such as working memory, attention, perception, and language. Recently, there is an increasing interest in large-scale resting-state network dynamics in health and disease, with the aim of identifying valuable biomarkers for diagnostic or prognostic evaluation.

Summary: MEG is a non-invasive, silent, and comfortable examination of functional neuroimaging modalities. MEG directly measures brain function with very high temporal resolution and good spatial resolution (Singh, 2014) and is widely used in neurological studies and clinical applications. With its millisecond temporal resolution, MEG is uniquely positioned to capture rapidly evolving neural dynamics.

1.3 Epilepsy

Epilepsy is one of the most common neurological diseases worldwide, affecting around 1% people of all ages around the world (**WHO**, 2019). It is characterized by recurrent seizures or unusual behaviors, sensations and sometimes loss of awareness arising from abnormal discharges of a large population of hyperexcitable neurons. It carries neurological, cognitive, psychological and social consequences and accounts for a significant proportion of the world's burden of disease. Due to the lack of literacy, epilepsy is misunderstood, discriminated against and socially stigmatized in many countries. People with epilepsy and their families suffer from stigmatization and discrimination and often encounter serious difficulties in education, employment, and marriage, especially in underdeveloped regions.

It is generally accepted that an imbalance between excitation and inhibition is the primary mechanism of transition from normal brain function to seizures (Sumadewi et al., 2023). Preclinical (fundamental) studies in epilepsy focus on the molecular, anatomical and cellular physiological changes involved in its development (epileptogenesis) and its seizure initiation (ictogenesis) (Kramer et al., 2012, Devinsky et al., 2018). Clinical studies have shifted interest from epileptiform discharges of a foci to epileptogenic networks of multiple functionally integrated cerebral regions. Both share the goal of revealing the causes of abnormal hyper-synchronization in neural activity to optimize diagnostic and therapeutic strategies for epilepsy.

1.3.1 Epidemiology

Epilepsy affects 65 million people worldwide of all ages, sexes, races, and income groups (Devinsky et al., 2018). More than 5 million new epilepsy cases are diagnosed every year, and the number is expected to increase further as the life expectancy rises worldwide.

1.3.1.1 Etiology

In 2017, the International League Against Epilepsy (ILAE) suggested classifying the etiology of epilepsy into six categories (Scheffer et al., 2017):

① **Structural etiology:** The structural etiology refers to the structural abnormalities that can be diagnosed by neuroimaging methods and that are supposed to be the cause of the patients' seizures. The structural abnormalities should be consistent with clinical evidence, such as clinical symptoms and neurophysiologic evaluation (EEG or MEG). Hippocampal sclerosis with mesial temporal lobe seizures is a typical example of a structural etiology. Other structural etiologies include tumors, stroke, and trauma.

② **Genetic etiology:** According to the definition by the International League Against Epilepsy (ILAE), genetic epilepsies result directly from a known or presumed genetic mutation in which seizures are a core symptom of the disorder (Scheffer et al., 2017). Genetic epilepsy may originate from monogenic or complex inheritance. Monogenic inheritance refers to pathogenic variants with either inherited or de novo (not inherited) origin of the epilepsy. In contrast, complex inheritance implies that variants in multiple susceptibility genes (with or without environmental involvement) contribute to epileptogenesis but are insufficient to cause epilepsy solely. Therefore, a genetic etiology does not equate to inherited. For example, more than 80% of Delaware syndrome, a severe developmental epileptic encephalopathy (DEE), are caused by pathologic variations of the *SCN1A gene*, however many of which are de novo mutations (not inherited) (Rastin et al., 2023).

③ **Infectious etiology:** The central nervous system infection is one of the major risk factors and the most common etiology of epilepsy, especially in some underdeveloped regions. Infectious etiology is defined as a patient with epilepsy suffering from seizures that are directly resulting from a known infection. In this case, neuroinfectious factors may contribute to seizures by damaging the brain

in a direct or indirect manner. For example, in neurocysticercosis, seizures are induced by cerebral damage (directly) and immune/inflammatory-mediated response in the infected brain tissue (indirectly) (Czuczwar, 2022).

④ **Metabolic etiology:** The concept of a metabolic epilepsy relates the etiology of epilepsy to known or presumed metabolic disorders in which seizures are a core symptom of the disorder (Scheffer et al., 2017). Common examples include porphyria, uremia, aminoacidopathies, or pyridoxine-dependent seizures. Most metabolic epilepsies will have a genetic defect, promoting epileptogenesis indirectly by negatively impacting cellular or organ function (i.e. cellular degeneration and demyelination) (Czuczwar, 2022).

⑤ **Immune etiology:** Patients with epilepsy of unknown origin, yet being seropositive for neural specific antibodies and showing evidence of autoimmune-mediated CNS inflammation can be classified as having an immune etiology. With the greater availability of antibody testing, the diagnosis of autoimmune encephalitis is gradually increasing, and studies have shown that autoimmune epilepsy accounts for about 5-7% of all epilepsies (Czuczwar, 2022, Husari et al., 2019). The accurate identification of this etiologic subgroup is critical to treatment, as autoimmune epilepsy requires immunotherapies rather than conventional antiepileptic medications.

⑥ **Unknown etiology:** It means that the cause of the epilepsy remains unclear. Identifying the etiology of epilepsy depends largely on our current knowledge of epilepsy and the tools and methods available for epilepsy evaluation. It varies across countries and regions still, due to level of development and health care setting differences.

1.3.1.2 Incidence and prevalence

Around 7.6 per 1000 persons have epilepsy during their lifetime, nearly 80% of them live in low- and middle-income countries (LMIC) (**WHO**, 2019). Fiest et al. showed in a systematic review and meta-analysis that the pooled incidence rate of epilepsy was 61.4 per 100 000 person-years (Fiest et al., 2017). Compared with 48.9/100,000 person-years in high-income countries, the incidence of epilepsy is higher in middle- and low-income countries, at 139.0 per 100,000 person-years. In an epidemiological study, Maloney et al. found that the incidence of epilepsy demonstrates a bimodal distribution according to age with peaks in the youngest individuals and in those over 60 years of age (Maloney et al., 2020) (**Fig.2**). The overall lifetime prevalence of epilepsy is 7.60 per 1000 population (Lifetime prevalence: The risk of having epilepsy at some point during the lifetime; it is used to estimate the cumulative incidence of epilepsy.); in low- and middle-income countries it is 8.75 per 1000 population, higher than in high-income countries with 5.18 per 1000 population. (Point prevalence: The number of cases of active epilepsy cases, divided by the target population on that day (active cases per 1000 persons).); in middle- and low-income countries, it is 6.68 per 1000 population higher than in high-income countries with 5.49 per 1000 population.

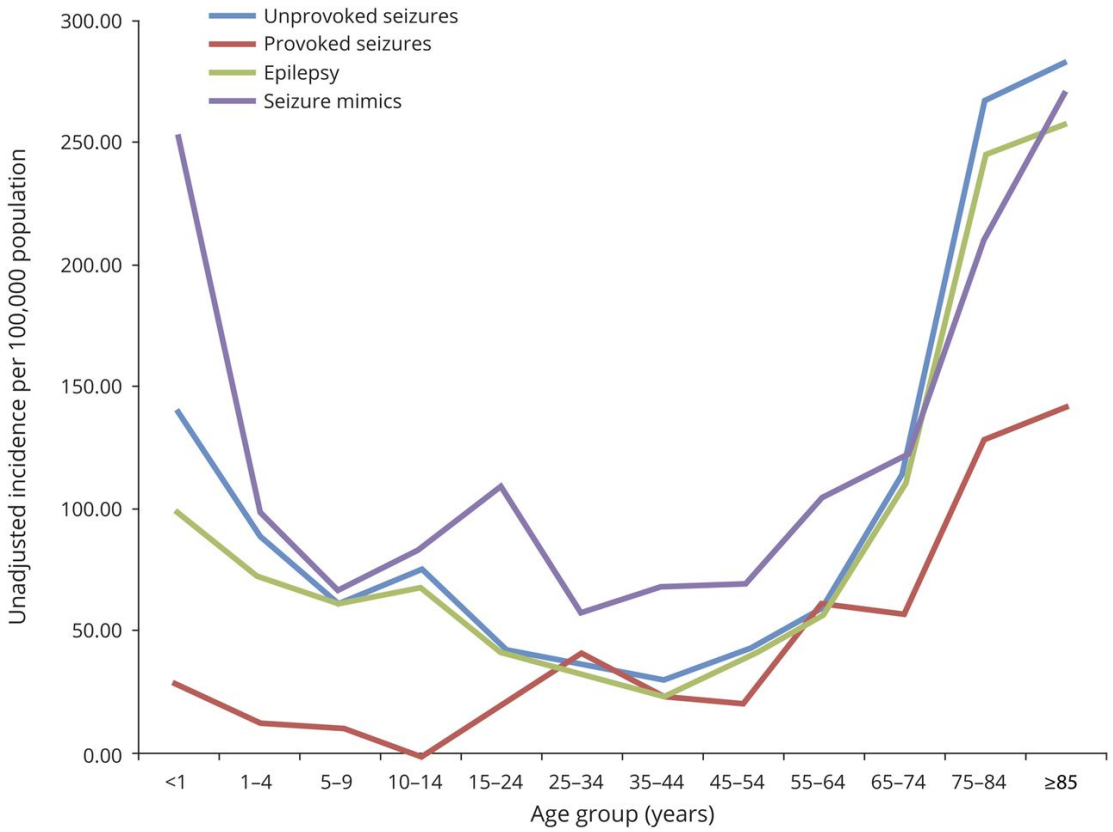

Fig.2. Unadjusted annual incidence. Unadjusted age group–specific annual incidence for definite and probable first unprovoked seizures, first provoked seizures, new diagnosis of epilepsy, and seizure mimics per 100,000 population during calendar year 2017(Maloney et al., 2020).

In a burden of epilepsy study, Beghi et al. reported similar findings that the highest prevalence of idiopathic epilepsy (hereditary or no other etiology found at diagnosis) was found in low- and middle-income countries like eastern, western, and southern Saharan Africa, Central Asia, central Andean Latin America, and Southeast Asia (Collaborators, 2019) (**Fig.3**). Both prevalence and incidence are higher in low-income and middle-income countries (LMICs) than in high-income countries. It might result from a greater exposure to perinatal risk factors, higher rates of infections and traumatic brain injury (TBI), the different structure of populations (demographic distribution) and variations in treatment in LMICs. No reliable evidence supports gender differences in incidence and prevalence, although some studies have demonstrated a slightly higher incidence in males, probably owing to concealment of the condition in

women driven by cultural or customary reasons, i.e. once diagnosed with epilepsy they suffer from discrimination in marriage and employment.

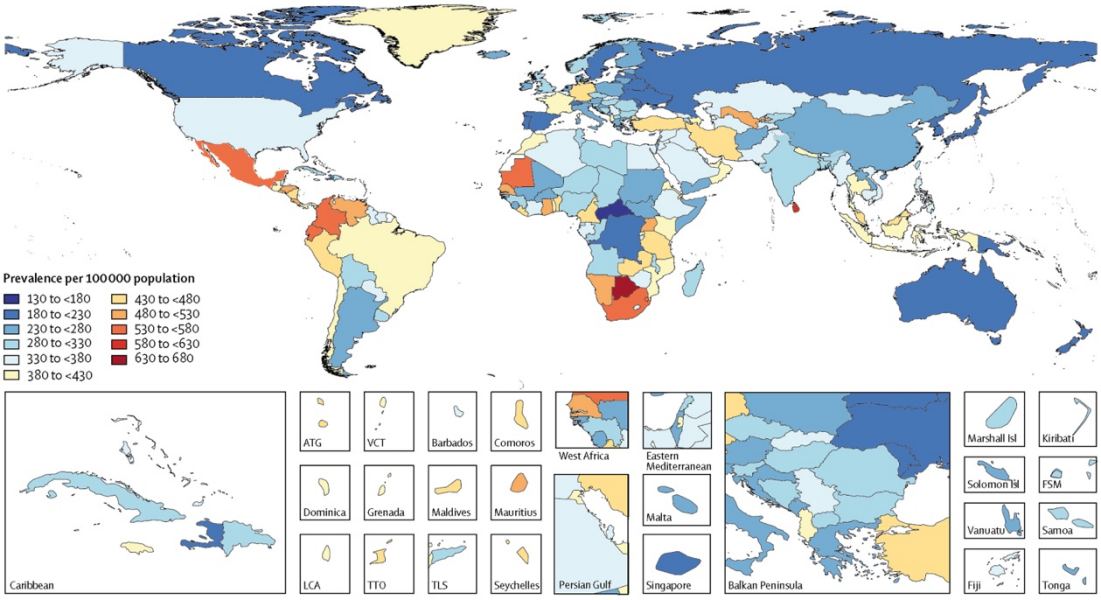

Fig.3. Age-standardized prevalence per 100 000 of idiopathic epilepsy for both sexes, 2016 (Collaborators, 2019)**.**

1.3.1.3 Mortality

Epileptics have a three times higher risk of death compared to the general population (Watila et al., 2018). Deaths attributed to epilepsy or seizures can be classified into direct causes (i.e., drowning, motor vehicle accidents, falls and burns, sudden unexpected death in epilepsy, status epilepticus) and indirect causes (i.e., aspiration pneumonia, suicide, and adverse effects of ASDs or psychiatric drugs, such as obesity and cardiovascular effects) [19]. Epilepsy in low-income and middle-income countries (LMIC) carries a significantly greater mortality than in high-income countries (HIC) (**WHO**, 2019). Unfortunately, in low- and middle-income countries, mortality often results from preventable causes such as lack of medical facilities, status epilepticus and drowning (Levira et al., 2017).

1.3.2 Diagnosis and classification of epilepsy

The precise and specific classification of epilepsy is essential for its treatment and management. Based on the suggestions from the International League Against Epilepsy in 2017, classification is achieved at three levels: seizure type, epilepsy type, and epilepsy syndrome, and at each level, the etiology and complications should be as clear as possible (with important implications for therapy) (Scheffer et al., 2017).

1.3.2.1 Seizure type

Based on characteristics of the onset, seizure types are categorized as focal, generalized, and unknown. (**Fig.4**)

- **Focal seizures** originate within neuronal networks limited to one cerebral hemisphere. According to the awareness level (retained or impaired awareness) and the earliest and most dominant manifestation (motor or non-motor), focal seizures will be further classified.
- **Generalized seizures** arise within some rapidly engaged and bilaterally distributed cortical networks, which can involve cortical and subcortical structures, but not necessarily the entire cortex. Generalized seizures are subdivided into motor and non-motor (absence) seizures (Thijs et al., 2019).
- **Unknown seizures**, a common scenario includes someone suffering from convulsions without clearly clinical evidence for a focal or a generalized onset.

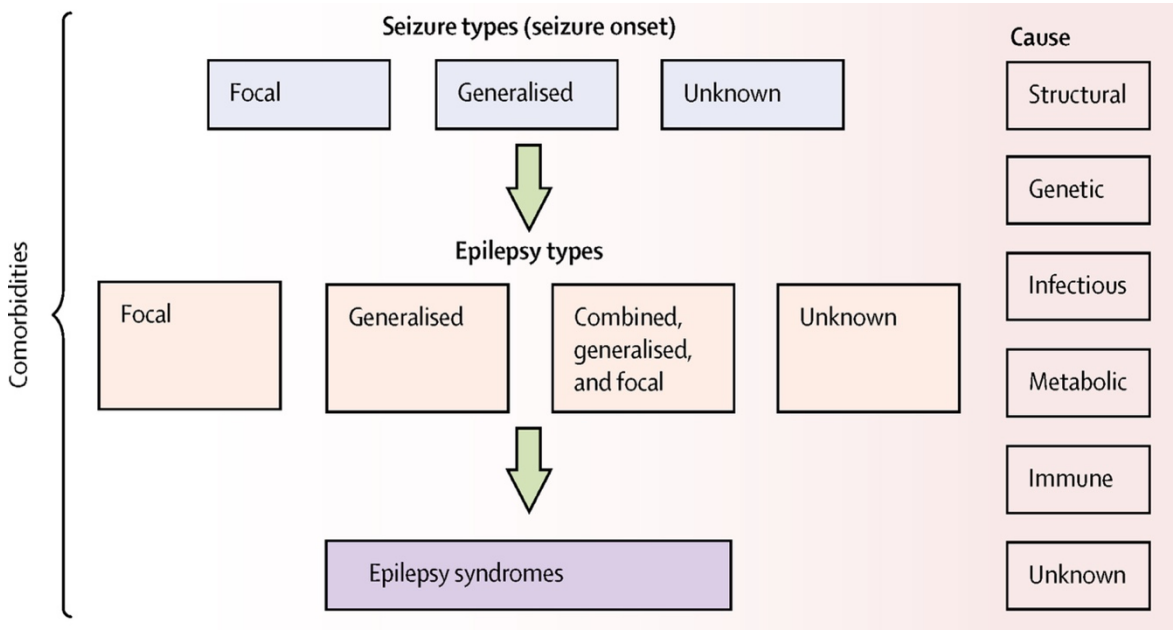

Fig.4. Classification of seizures (Scheffer et al., 2017).

1.3.2.2 Epilepsy types

There are four categories of epilepsy types: focal, generalized, combined generalized and focal, and unknown, differentiated on clinical grounds (i.e. seizure type) and neurophysiological evidence (i.e. EEG findings) (**Fig.5 & Fig.6**).

- **Focal epilepsies:** typical focal epileptiform discharges on interictal EEG as well as focal seizures contribute to the diagnosis.
- **Generalized epilepsies:** for a diagnosis, the patient would typically show generalized spike-wave activity on EEG and suffer from Generalized-onset seizures.
- **Combined Generalized and Focal Epilepsies:** patients who have both generalized and focal seizures, and the ictal and interictal electroencephalographic manifestations of generalized spike-wave or focal epileptiform discharges (*but epileptiform activity is not strictly required for diagnosis*).
- **Unknown:** a diagnosis of epilepsy is established, but it can be limited by

insufficient clinical evidence that prevents the identification of a specific type of epilepsy. The reasons behind vary, for example, the EEG is inaccessible, or the EEG examinations are uninformative.

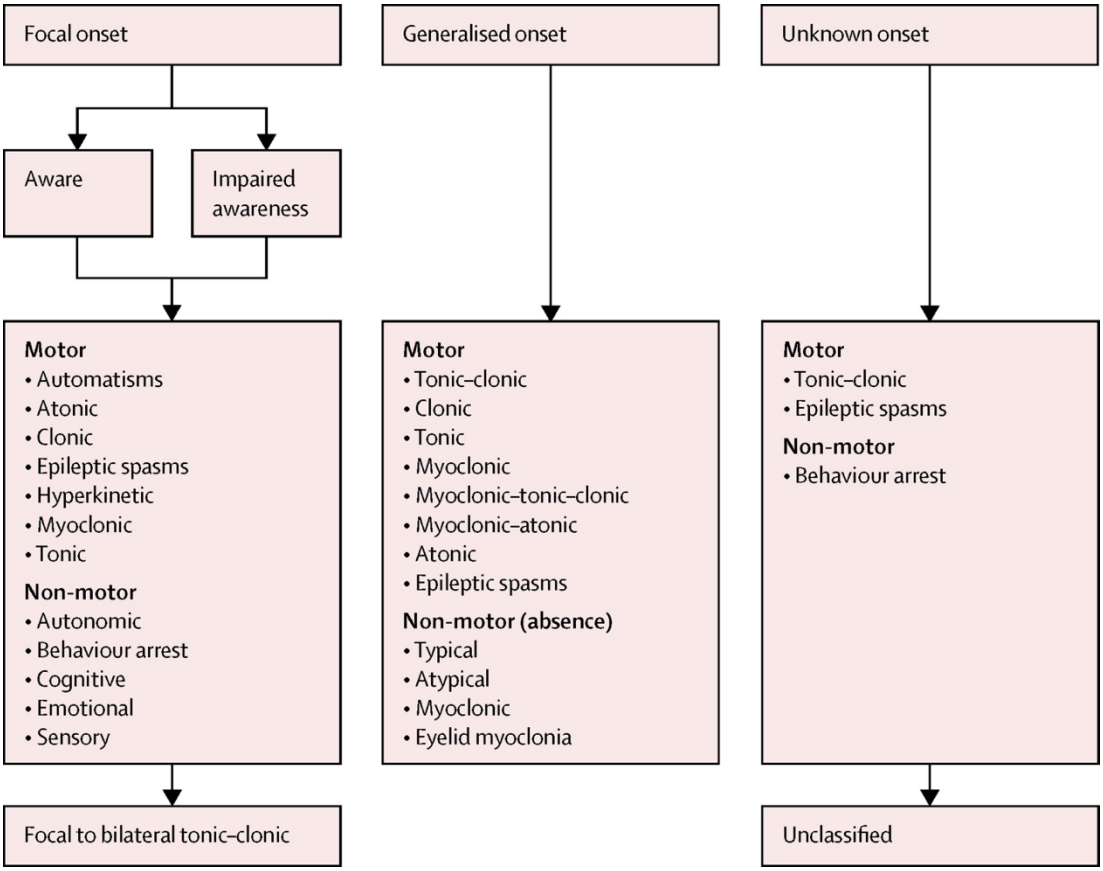

Fig.5. Classification of epilepsies (Scheffer et al., 2017).

1.3.2.3 Epilepsy syndrome

Epilepsy syndrome is the diagnosis with the highest level of reliability, which is derived from a group of clinical features including age of onset and remission, seizure type, comorbidities, EEG and imaging features. In our study, all patients enrolled had a diagnosis at the level of epilepsy syndrome.

Generalized epilepsy, unlike focal epilepsy with a well-defined epileptogenic focus, involves widespread and synchronous activation across multiple brain regions, making it a quintessential example of a network disorder. Understanding the underlying mechanisms of generalized epilepsy requires examining how brain dynamics are altered at a global level, rather than just

focusing on localized dysfunction. This research aims to explore the dynamic brain states in generalized epilepsy, which could provide insights into the broader functional disruptions occurring across the brain. By studying this type of epilepsy, we seek to uncover how brain networks transition between different states during seizures, offering a comprehensive view of the disorder's impact on brain function.

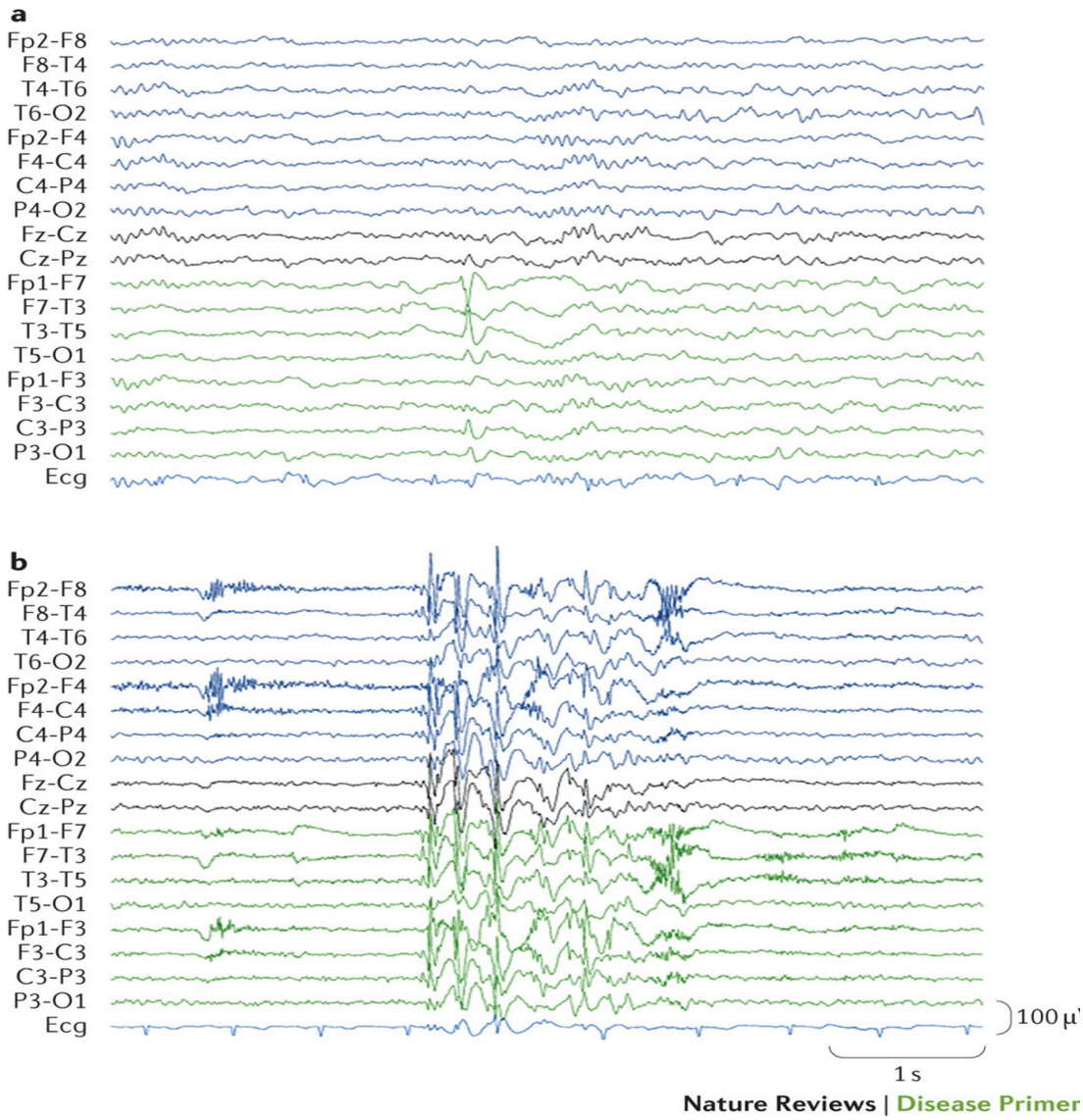

Fig.6. Interictal abnormalities detected using EEG. a. focal epileptiform discharge (FED)；b. generalized epileptiform discharge (GED) (Devinsky et al., 2018)

Summary: Epilepsy, a prevalent neurological disorder, affects approximately 1%

of the global population, leading to recurrent seizures and diverse neurological, cognitive, and social challenges. Given its high incidence and mortality rates, often stigmatized and misunderstood, significantly impairs the lives of affected individuals and their families, particularly in underdeveloped regions. The pathophysiology of epilepsy is attributed to an imbalance between neuronal excitation and inhibition, prompting research to focus on both the molecular and network-level mechanisms underlying seizure development. Recent clinical studies emphasize the importance of understanding epileptogenic networks across multiple brain regions, rather than isolated epileptiform discharges, to improve diagnostic and therapeutic strategies. In this thesis, a new approach to studying epileptic networks with high temporal resolution is proposed to capture transient brain state fluctuations, aiming to better explain alterations of dynamic characteristics of epileptic networks.

1.4 Diagnostic and therapeutic challenges in generalized epilepsy

1.4.1 Overview of generalized epilepsy

Approximately one-third of epileptic patients suffer from seizures that are unresponsive to medications, resulting in drug-resistant epilepsy (DRE) (Guery et al., 2021). Surgical removal of MRI-identified epileptogenic lesions is the standard treatment option for drug-resistant focal epilepsy. Currently, the most serious diagnostic and therapeutic challenges arise from drug-resistant generalized epilepsy. MRI evaluation may reveal disappointing results in such patients, e.g. these patients may have widely distributed lesions or unrecognized lesions. These patients are unable to benefit from surgical treatment and the therapeutic guidance provided by MRI-based epileptogenic lesion identification. Therefore, it is necessary to integrate structural imaging with other functional imaging to reveal the pathophysiology in these cases. With significantly higher temporal resolution than functional magnetic resonance imaging (fMRI) and less affected by volume conduction effects in comparison to electroencephalography (EEG), magnetoencephalography (MEG) has received increasing attention for MRI-negative epilepsy studies (Li Hegner et al., 2018).

1.4.2 MEG and brain networks in generalized epilepsy

MEG, as a non-invasive imaging tool, was clinically applied firstly for localizing epileptiform discharges to identify epileptogenic foci. A systematic review study stated that in terms of localizing seizure foci, the performance of MEG is comparable to intracranial EEG (Zhang et al., 2014). The knowledge about the nature of epilepsy is still evolving and has developed from the conceptualization of a discrete focus, the epileptogenic zone, to the idea that epilepsy is a typical neural network disorder (Girardi-Schappo et al., 2021, Litwinczuk et al., 2022).

Functional connectivity (FC) is a correlation measure between different cortical regions to characterize neural networks in healthy and diseased individuals.

The application of graph theory to neural network studies has provided researchers with a powerful tool to quantify neural network features such as node strengths, path lengths, clustering coefficients, synchronicity, and centrality. Our previous study on MRI negative generalized epilepsy found that patients showed the widespread increased connectivity in resting state networks (e.g. motor networks, in the medial frontal and temporal cortex) compared to Healthy controls (Elshahabi et al., 2015). Interestingly, Zhang et al, in another resting-state network study, found that regions contributing to the pathogenesis of MRI-negative global epilepsy may show abnormal hub characteristics (Zhang et al., 2011). Most of these studies focused on network features such as path lengths and clustering coefficients, which may provide some clues but may be insufficient to fully understand the relationship between network dynamic organization and brain function in interictal epilepsy. Therefore, further research focusing on other network characteristics are necessary to provide insights into the complex organization between interictal brain networks.

1.4.3 Brain states in generalized epilepsy

The human brain comprises a set of distinct and highly sophisticated networks dealing with specific functions (Pedersen et al., 2018). The ongoing dynamics of these networks exhibit condition-dependent self-organization, traversing stable, "quasi-stable," high or low activations and transient configurations, known as brain states (Deco et al., 2019). The hierarchically organized brain states underlie cognition and behavior. Altered brain state dynamics at test and task resulting in neurological dysfunction has been described in temporal lobe epilepsy (TLE) (He et al., 2018, Banjac et al., 2021, Girardi-Schappo et al., 2021). Compared to the assessment of static functional connectivity within a time window, such rapidly fluctuating brain network (state) dynamics may better contribute to a deeper understanding of epilepsy. We infer that alterations of brain state dynamics in generalized epilepsy can be found and contribute to the

symptomatology. Therefore, in this thesis, we utilize a brain state identification method with high temporal resolution to characterize brain state dynamics in generalized epilepsy.

Summary: Epilepsy is a neurological network disorder. Over the past decade, brain network studies based on static functional connectivity have deepened our understanding of epilepsy. However, our brain is a dynamic system with rapidly changing functional demands, and static network studies may not be sufficient to help us view the full picture of epilepsy. In this thesis, we aim to compare the differences in brain state dynamics between healthy controls and patients with generalized epilepsy using an analytical method that can capture the transient brain network dynamics (brain states).

1.5 Hypothesis：

To adapt to a rapidly changing external and internal environment, the brain needs to constantly engage different brain networks for sensory, motor, cognitive demands, and autonomic regulation of body functions. Since epilepsy is regarded as a network disease, there might be state-dependent network differences between generalized epileptic patients and healthy controls:

1) the dynamic parameters describing brain states (FO, AR, DT and TP), might differ between generalized epileptic patients and healthy controls in different conditions (e.g., Eyes close vs Eyes open; Rest vs Task).

2) compared to healthy participants, transitions between brain states may exhibit abnormalities in generalized epilepsy patients.

3) classification models trained with features of dynamical parameters of brain states might effectively discriminate between generalized epilepsy and healthy controls.

2 Methods

2.1 Participants

Twelve patients with generalized epilepsy (MRI-Negative) were recruited from the Department of Neurology and Epileptology, Hertie Institute for Clinical Brain Research, University of Tübingen. The diagnosis was determined by experienced epileptologists based on a comprehensive evaluation, including neurological history and examination, interictal and ictal scalp video EEG, and MRI. Sixteen healthy participants were enrolled in the study as healthy controls excluding any history of neurological disorders, head trauma and contraindications to MRI. All patients and healthy controls enrolled in MEG and MRI recordings were free of metal implants to enable high-quality data acquisition. This study was approved by the Ethics Committee of the University of Tübingen and carried out in accordance with the Declaration of Helsinki. All participants provided written informed consent. Due to the effect of head movement on the MEG signal, eventually data from 11 patients (9 Females,2 males) and 12 healthy subjects were used for follow-up analyses (One patient and four healthy participants were excluded). The demographic characteristics of epilepsy patients are described in **Table 1**.

2.2 MEG recording

Data under four different conditions were collected (sampling rate 2400 Hz) in upright position at the MEG Center of the University of Tübingen (275-channel system, CTF Inc., Vancouver, Canada). Briefly, MEG data were collected in a factorial design with two-by-two different conditions (eyes open and eyes closed) combined with two different states (resting and task). The specific conditions were as follows: I. eyes closed; II. eyes open; III. eyes closed with Fibonacci counting and IV. eyes open with Fibonacci counting. Participants were instructed to relax and minimize head movement during the entire recording. For the resting-state recordings (I and II), participants were instructed not to think about anything in particular. During task-state recordings (III and IV),

participants were instructed to perform Fibonacci counting silently in their minds. For all conditions requiring eyes open, participants were instructed to fixate on a white cross located at the center of a black screen positioned in front of them.

2.3 Head Anatomy

For each participant, a high-resolution (voxel size = 1 × 1 × 1 mm) T1-weighted whole-head structural image (3D-MPRAGE, TR 2.3 s, TE 3.03 ms, FA 8°) was acquired on a Siemens Prisma 3T scanner (Siemens AG, Erlangen, Germany) with a 64-channel head coil. Then, we processed the head anatomical data acquired with MRI according to the methods recommended by Hegner et al (Li Hegner et al., 2018). Briefly, individual cortical surfaces were reconstructed using the FreeSurfer software package (surfer.nmr.mgh.harvard.edu/). The cortical spatial alignment among subjects was achieved by warping each hemisphere of the individuals into a standard spherical space, allowing the spatial correspondence mapping (normalization) of cortical regions. Next, the cortical surfaces (reconstructed by Free-Surfer) of each participant were segmented into 1,002 common vertices per hemisphere (2004 vertices in total for the whole brain) with SUMA (afni.nimh.nih.gov/download/). A template of the cortical mesh surface for visualization was acquired by resampling the surface with a fsverage template (average brain mesh from Freesurfer) and SUMA (ld = 10). Subsequently, the cortical mesh of each participant was realigned to the MEG sensor space (CTF coordinate system).

2.4 Data Processing and Source Analysis

MEG data preprocessing and analysis was performed using MATLAB (version: R2023b, https://www.mathworks.cn/downloads/) built-in algorithms and the open-source neural signal processing toolbox Fieldtrip (https://www.fieldtriptoolbox.org/). Briefly, for each participant, we band-pass filtered the data between 2 and 40 Hz as well as computed the standard deviation (STDs) for each channel. Subsequently, we calculated the median and median absolute deviation (MAD) of STDs across channels at the individual

and cross-participant level for outlier detection, to remove channels and participants with low signal-to-noise ratio (SNR). Independent component analysis was applied to further reject heart and eye movement artifacts. The data was then downsampled to 100Hz.

Next, the time domain data at the sensor level were projected to source space with the linear constrained minimum variance beamforming (LCMV). For each participant, a “single-shell” approach was applied to generate the volume-conduction head model. The lead field matrices and common spatial filter (Tikhonov regularization: λ = 5%) were then calculated for the 2004 cortical vertices.

2.5 Brain states and its dynamic features

Following Cornblath et al. (Cornblath et al., 2020), the source-level time series data of all subjects were concatenated to generate a matrix $\mathbf{D}$ with $\mathbf{N}$ rows (observations) and $\mathbf{P}$ columns (features). Here, $\mathbf{P}$ is the number of source (2004 brain regions), and $\mathbf{N}$ is the number of observations for each condition (5 min x 60 s x 100 Hz) x 4 conditions x participants (11 patients, 12 healthy controls, 23 participants in total), summing up to $\mathbf{N}$ = 2760000. Z-scores were applied to $\mathbf{D}$ along the columns such that each brain region resulted in a mean of 0 and a standard deviation of 1. All data in $\mathbf{D}$ were squared to avoid effects of sign flipping on subsequent cluster analysis. Then K-mean clustering was applied (20 repetitions) to identify brain activation patterns (brain states), using the Pearson correlation as a distance measure. Since we were interested in cross-group comparisons of brain state dynamics between epilepsy patients and healthy controls, it was important to use brain states shared by all subjects. We tested clustering from k = 4 to k = 10. At k = 10, there were partial loss of states in some participant. Therefore, we used the brain states identified with k=9 for follow-up dynamics analyses (**Fig.7**).

Next, we characterized brain state dynamics using fractional occupancy (FO), dwell time (DT), and appearance rate (AR) and transition probability (TP).

Briefly, (1) fractional occupancy, the percentage of a certain state assigned to a given condition, (2) dwell time, the average duration (in milliseconds) of continuous runs for a given state, (3) appearance rate, the number of times that a given state is present per minute were computed. Furthermore, we defined (4) the transition probability between state i and state j as the probability that after state i, state j becomes the next new state. FO, DT, AR, and TP were computed separately for each of the four conditions, and the averaged FO/DT/AR/TP was obtained by averaging across conditions for each subject. These brain dynamics metrics were compared between epileptic healthy groups using the Wilcoxon rank-sum test and corrected for multiple comparisons using Benjamini – Hochberg (BH) with a false discovery rate (FDR) of 0.05.

2.6 Entropy calculation

To quantitatively describe the transition dynamics between brain states, we calculated the entropy $\boldsymbol{E}$ of each brain state i, based on the transition probabilities. Larger entropy values indicate that transitions between brain states are less predictable and more complex, whereas smaller entropy values suggest that these transitions are more predictable and less complex (Richman et al., 2000, Smith et al., 2014). Such increased complexity and decreased predictability suggest a greater propensity for information processing of the brain and reflect the accessibility of different brain states and the capacity for transitions between states (Saxe et al., 2018, Keshmiri, 2020).

$$\boldsymbol{E}(x_i) = -\sum_{ij} p(x_{ij}) log_2\, p(x_{ij})$$

$p(x_{ij})$ is the probability of transition from state i to state j.

2.7 Multivariate classification

Support vector machine (SVM) classification algorithm based on radial basis function kernel (RBF) was applied for multivariate pattern classification to investigate MEG signal differences between epilepsy and healthy participants.

The variables describing the brain state dynamics (FO, DT, AR and TP) were used to construct feature vectors or feature matrices under each independent condition (**IC**) and average condition (**AC**). Briefly, For **IC**, the FO, DT, AR or TP of 9 brain states from each participant were used to create individual feature vectors for group-level classification, whereas for **AC**, these variables were averaged across conditions before classification. A 5-fold cross-validation was repeated 20 times to assess the performance of the classifiers. A permutation test was used to determine if classification accuracy exceeded chance levels. Patient and control labels were randomly reassigned to the classifier and the classification process was repeated 10,000 times. Classifier performance above chance level was considered statistically significant (two-tailed paired replacement t-test, $\alpha = 0.05$).

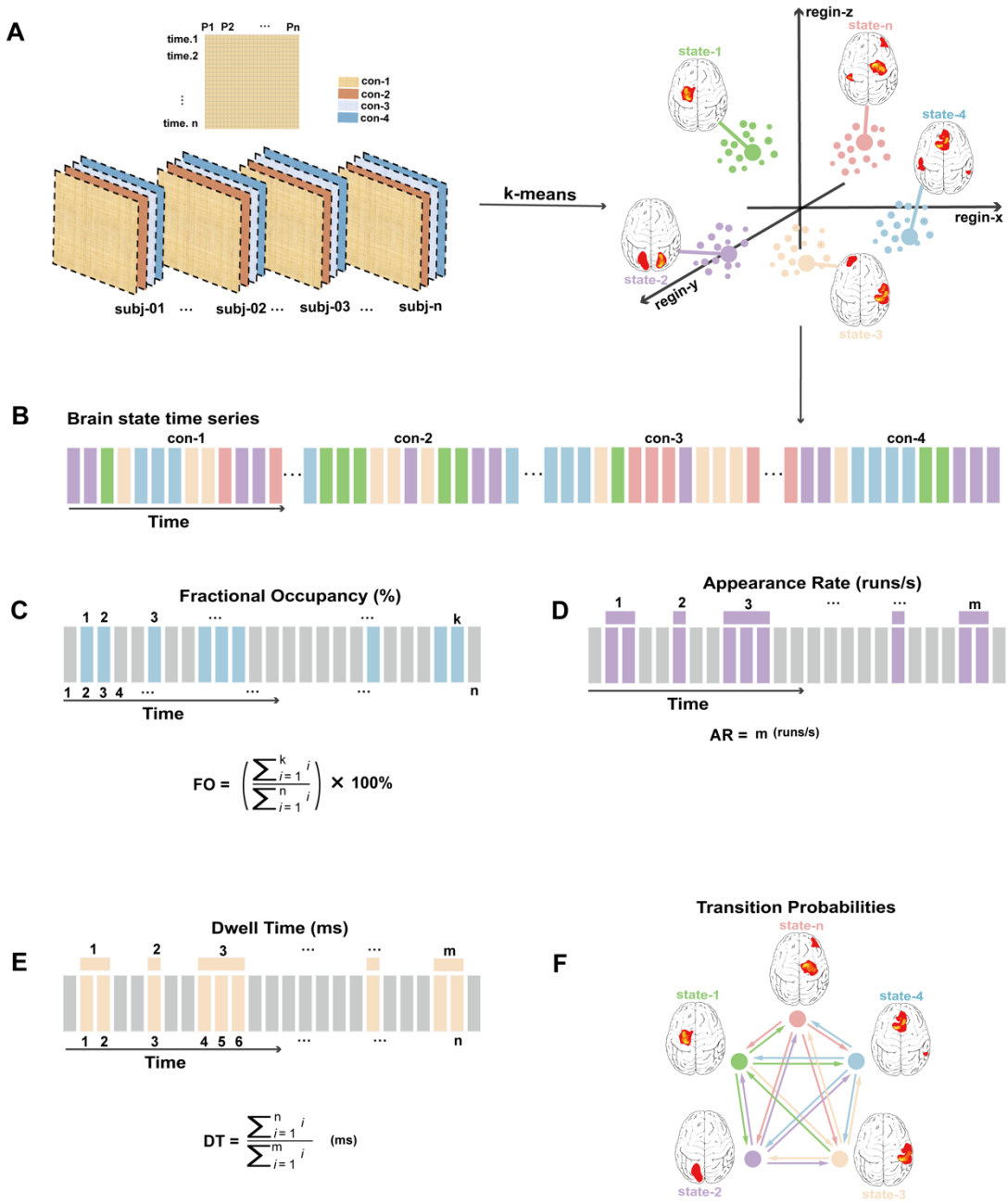

Fig.7. k-means clustering and computing brain dynamic state parameters. A) constructing the data matrix **D** by concatenating the data from all the participants (Epileptic patients and healthy controls). For matrix **D**, each row **N** is the number of observations (time points), and each column **P** is the number of sources (2004 brain regions). The k-means algorithm identified different clusters, and the centroids of these clusters were defined as brain states. B) Time series of brain states. C) Fractional occupancy (FO), D) appearance rate (AR), E) dwell time (DT), and F) transition probability (TP) was calculated for each state and for each participant. (Image adapted from references (Cornblath et al., 2020, Olafson et al., 2022))

3 Results

Table1. Demographic characteristics of generalized epilepsy patients	
	Patients (11 in total)
Age (years)	26.8 ± 5.6
Gender	9 Females, 2 Males
Age at onset (years)	13.4 ± 4.6
Epilepsy syndrome (%)	
CAE	9.0%
JAE	45.5%
JME	27.3%
GGE	18.2%
Duration of disease(years)	13.4 ± 6.4

CAE = childhood absence epilepsy, JAE = juvenile absence epilepsy, JME = juvenile myoclonic epilepsy; GGE = genetic generalized epilepsy (unclassified). Values are n (%) or mean (range).

3.1 Nine transient brain states are shared between epileptic and healthy individuals.

We collected MEG data from 23 participants (11 generalized epilepsy patients, 12 healthy controls) under four conditions (2 at rest, 2 at task). K-means clustering was then used to categorize the integrated data matrix (combining data from all participants and conditions) into clusters of statistically similar and temporally recurring whole-brain spatial co-activation patterns, termed 'brain states'. Since we were interested in cross-group comparisons, the optimal number of clusters was determined as the maximum number of clusters that could be captured for all participants in all conditions. Overall, we identified nine brain states shared between generalized epilepsy patients and healthy controls across all conditions. **State1** left motor region, **State2** subcortical region, **State3** frontoparietal region, **State4** frontal region, **State5** right temporal-parietal region, **State6** right motor region, **State7** occipital region, **State8** left temporal-

parietal region and **State9** limbic region (**Fig.8**).

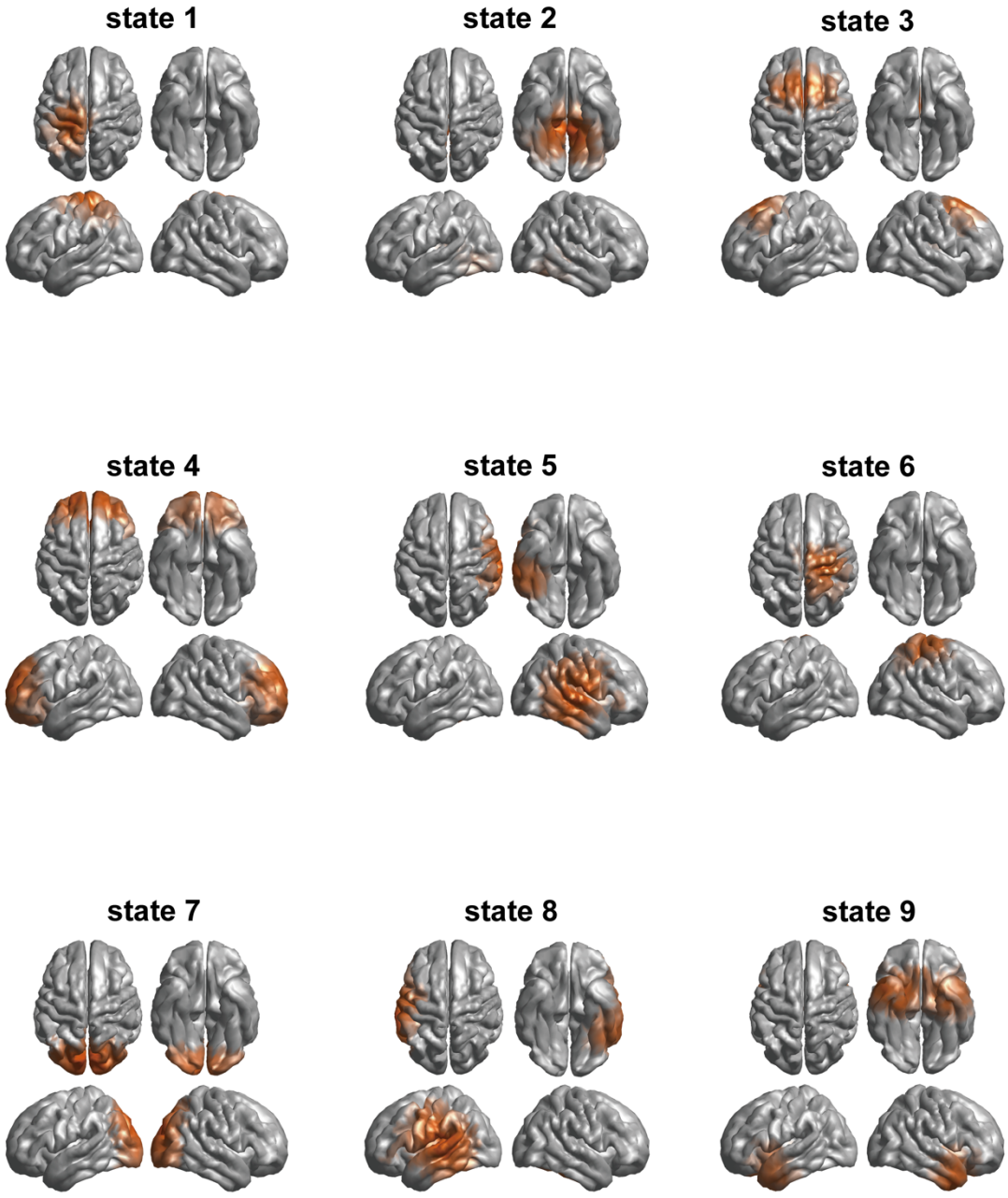

Fig.8. The k-means algorithm identified shared brain states between generalized epilepsy patients and healthy controls. We identified 9 spatial co-activation patterns (brain states) from the centroids of the clusters identified by the k-means algorithm. These patterns were shared across all conditions for all participants. **State1** left motor region, **State2** subcortical region, **State3** frontoparietal region, **State4** frontal region, **State5** right temporal-parietal region, **State6** right motor region, **State7** occipital region, **State8** left temporal-parietal region and **State9** limbic region.

3.2 Altered brain state dynamics in epileptic patients.

With large-scale brain states representing transient coactivation identified, we were interested in comparing the dynamics of brain states between epileptic and control participants. We introduced three metrics: Fractional Occupancy (FO), Dwell Time (DT) and Appearance Rate (AR) to quantitatively characterize the rich dynamics of brain states. Using Wilcoxon rank-sum test, we assessed whether these metrics differ between two groups (epilepsy vs. control) in the four independent conditions and averaged across conditions, respectively.

In independent condition IV (eyes open with Fibonacci counting), we found that compared to controls, epileptic patients showed significantly lower FO **(Fig.S1 D)** at state 6 (U-statistic: -2.5849, $p = 0.0097$, $p_{FDR} = 0.0438$) but higher FO at state 7 (U-statistic: 2.5849, $p = 0.0097$, $p_{FDR} = 0.0438$). No differences in FO were found between the two groups in cross-condition averaging (**Fig.9 A**).

Interestingly, significantly increased AR in epileptic patients at state 3 was observed both in independent condition III (eyes closed with Fibonacci counting, U-statistic: 2.9541, $p = 0.0031$, $p_{FDR} = 0.0282$, **Fig.S3 C**) and in cross-condition averaging (U-statistic: 2.8926, $p = 0.0038$, $p_{FDR} = 0.0343$, **Fig.9 C**). Regarding the DT measures, no between-group differences were found either in the four independent conditions (**Fig.S2**) or in the cross-condition averaging (**Fig.9 B**).

Notably, there were no significant differences observed between the two groups in the resting state (independent condition I and II) of FO (**Fig.S1 A, B**), DT (**Fig.S2 A, B**), and AR (**Fig.S3 A, B**). These results indicate that altered brain state dynamics in generalized epilepsy patients are more favorable to be captured during task execution.

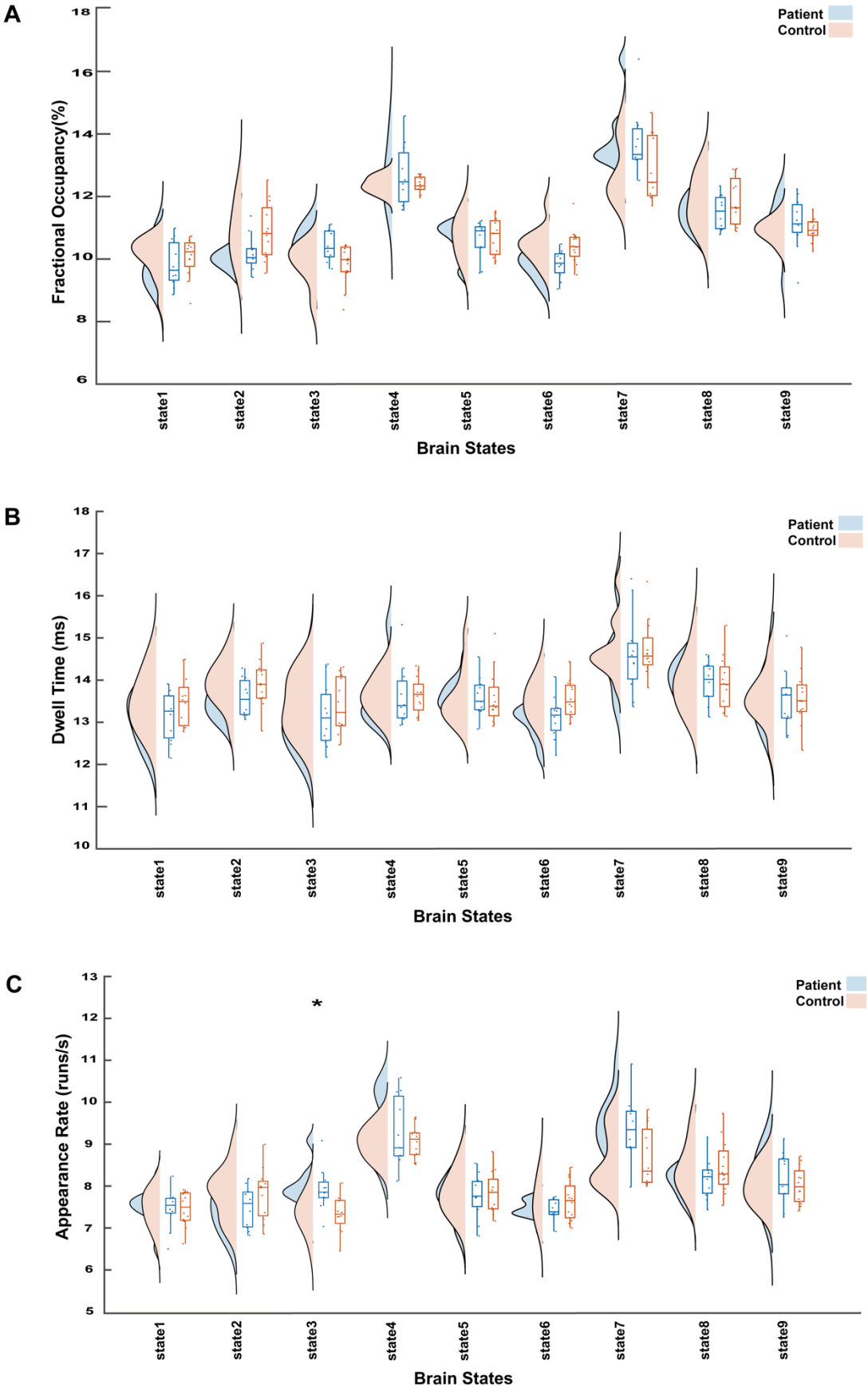

Fig.9. Brain state dynamics in epileptic and healthy participants. Distribution of

group-level fractional occupancy (A), dwell time (B), and appearance rate (C between generalized epilepsy patients and healthy controls for each brain state (averaged across all conditions). *p FDR <0.05 indicates that the P-value is less than 0.05 after multiple comparisons correction.

3.3 Altered brain entropy explains differences in brain state transitions between epilepsy patients and healthy participants.

The transitions between different brain states over time underlie our brain function. To explore whether these transitions are different in epileptic and healthy individuals, we quantified transitions between brain states using transition probabilities (TP) (**Fig.10 A**). We visualized state transitions at a threshold of 13.6 % (Three-quarter quartile of state transition probabilities averaged across participants and across conditions), with thicker lines representing a higher probability of transition (**Fig.10 B**). Additionally, we quantified the complexity and predictability of brain state transitions using entropy. Similarly, we assessed differences in TP and entropy between the two groups (epilepsy group and control group) in four independent conditions, and averaged across conditions, respectively, using the Wilcoxon rank sum test. Statistical analyses revealed a significantly lower entropy in the epilepsy group compared to healthy controls at state 5 (U-statistic: -2.8311, $p = 0.0046$, $p_{FDR} = 0.0415$), state 6 (U-statistic: -2.5849, $p = 0.0097$, $p_{FDR} = 0.0415$) and state 8 (U-statistic: -2.4618, $p = 0.0138$, $p_{FDR} = 0.0415$) when averaged across conditions (**Fig.10 C**). Notably, no differences in entropy between groups were found in the four independent conditions. These results suggest that brain state transitions in the epilepsy group were more regular (more predictable), whereas in the healthy control group were more flexible (less predictable), although we intuitively concluded that the network of brain state transitions was similar in the two groups.

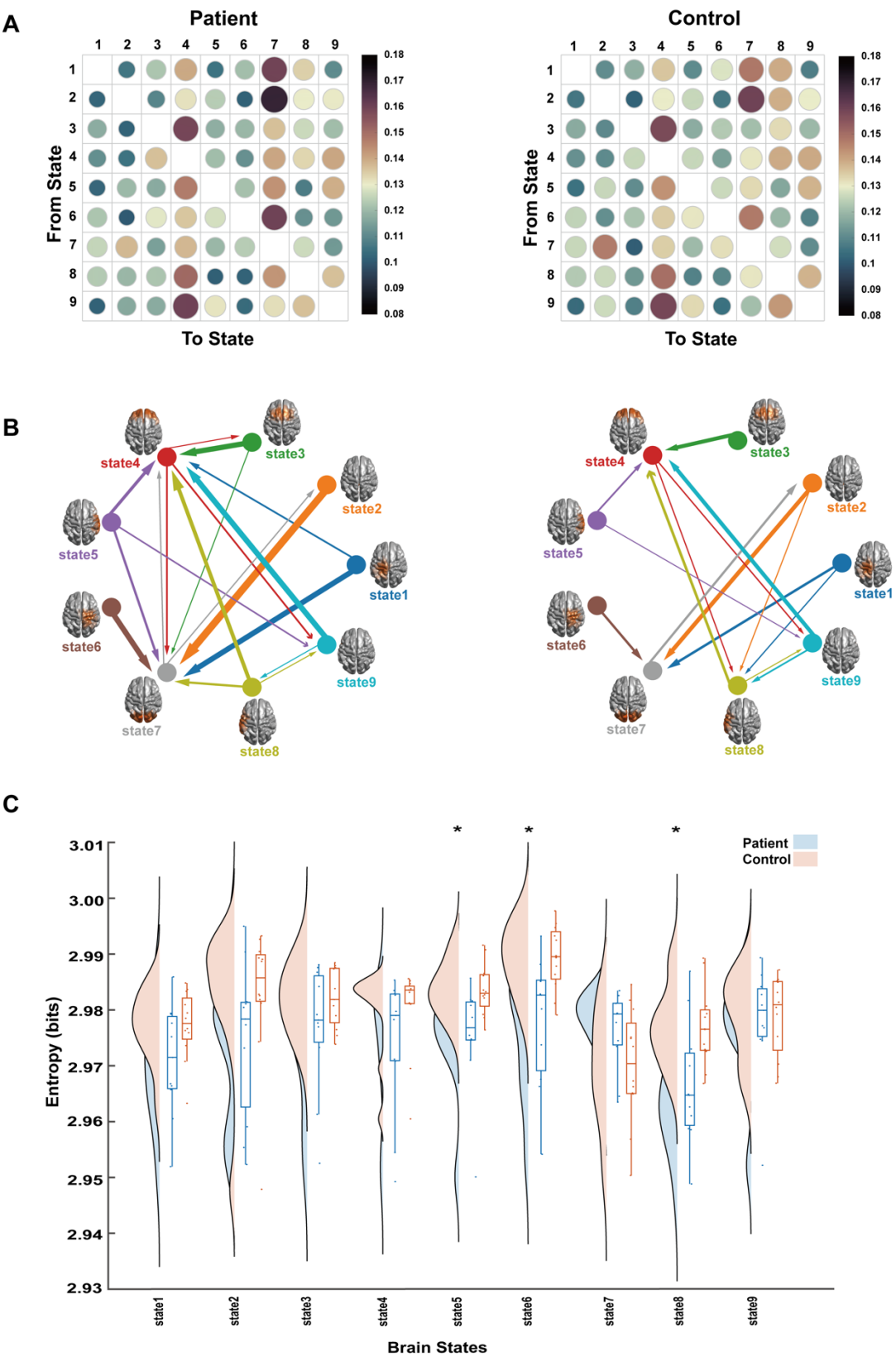

Fig.10. Brain state transitions in epileptic and healthy participants. A) brain state transition probability matrices of the global epilepsy group (left) and of the healthy control group (right) after averaged across all conditions. Matrix elements represent the probability of transitions between brain states after removing the

effects of autocorrelation. B) visualization of brain state transitions. Brain state transitions were visualized at a threshold of 13.6%, with a thicker line indicating a higher probability of transition (13.6% is the three-quarter quartile of the transition probability across all states for all participants). C) Entropy quantifies the complexity and predictability (flexibility) of each brain state transition. *p FDR <0.05 indicates that the P-value is less than 0.05 after multiple comparisons correction.

3.4 Multivariate analyses effectively differentiate epileptic from healthy participants.

Finally, the support vector machine classification algorithm was applied to evaluate the robustness of the parameters describing the brain state dynamics in differentiating generalized epilepsy patients from healthy controls. Five-fold cross-validation (20 repetitions) was performed to evaluate the accuracy of the classifier. A randomized permutation test with 10,000 permutations was performed to assess if classification accuracy exceeded the chance level (significance was established at less than 0.05).

Interestingly, FO performed well in classifying epilepsy and healthy groups in all four independent conditions and averaged across conditions: in condition I (acc = 71.68%, $p_{FDR} = 0.0231$), in condition II(acc = 72.08%, $p_{FDR} = 0.0233$), in condition III (acc = 72.24%, $p_{FDR} = 0.0249$), in condition IV (acc = 70.80%, $p_{FDR} = 0.0285$) (**Fig.S6 A**) and in averaged across conditions (acc = 71.96%, $p_{FDR} = 0.0276$) (**Fig.11 A**). Furthermore, the transfer matrix effectively differentiated the epilepsy group from the healthy control group in conditions I, III, IV (**Fig.S6 D**) and cross-condition averaging: in condition I (acc = 66.48%, $p_{FDR} = 0.0360$), in condition IV (acc = 68.50%, $p_{FDR} = 0.0440$) and in averaged across conditions (acc = 69.80%, $p_{FDR} = 0.0303$, **Fig.11 D**). Notably, no significant classification accuracy was observed in multivariate classification with DT (**Fig.11 C, Fig.S6 C**) and AR (**Fig.11 B, Fig.S6 B**) as features. These results indicate that TP and FO could be promising biological markers for characterizing altered brain dynamics in generalized epilepsy.

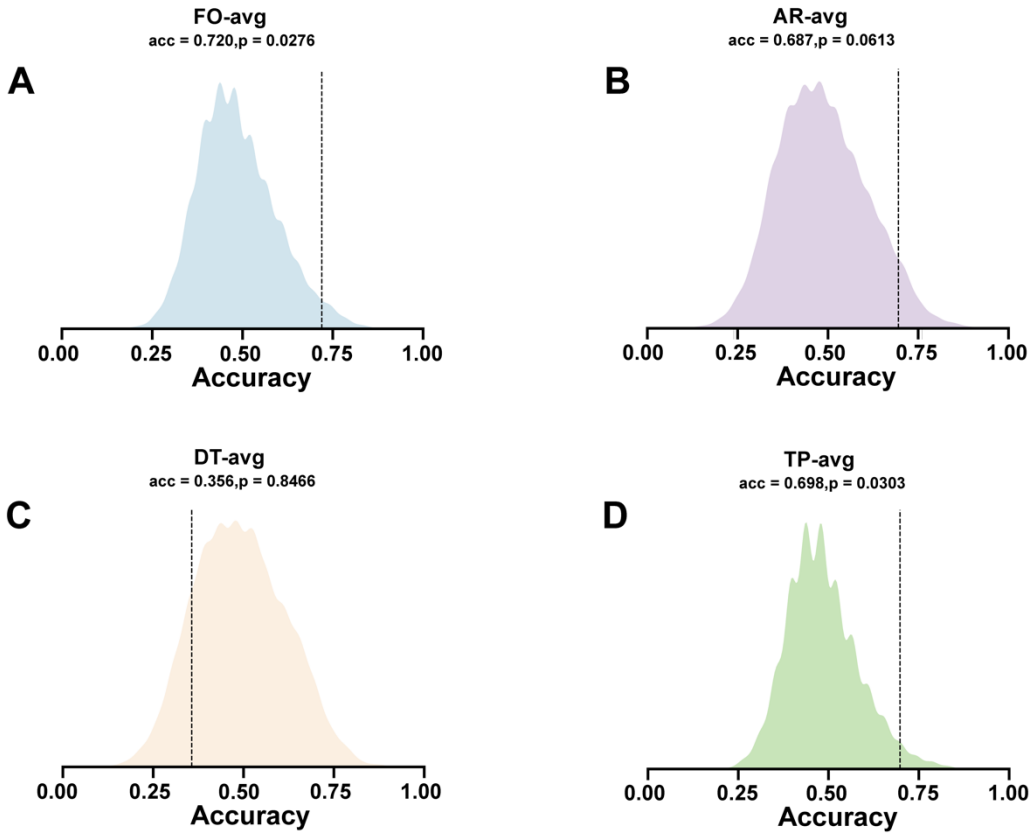

Fig11. Multivariate classification of brain state dynamic parameters. The brain state dynamics parameters (FO, AR, DT and TP) of each participant were averaged across conditions. These averaged parameters A) FO-avg, B) AR-avg, C) DT-avg and D) TP-avg were used as features for multivariate classification using support vector machines to determine their robustness in differentiating brain state dynamic differences between generalized epilepsy patients and healthy controls. The accuracy of the classification was determined with 5-fold cross-validation. A permutation test was performed to determine if classification accuracy exceeded chance levels. *p FDR <0.05 indicates that the P-value is less than 0.05 after multiple comparisons correction.

4 Discussion

Large-scale brain activation patterns (brain states) and its dynamic evolution underlie cognition and behavior. Here, brain states are defined as a set of distinct patterns of networks coactivated in space. Investigating brain state dynamics may contribute to our knowledge on epilepsy, however, less is known about the link between epilepsy and brain state dynamics. The present study aimed to compare brain state dynamics between healthy individuals and patients with generalized epilepsy during resting and task. Utilizing machine learning algorithms, we identified nine brain states shared by epileptic and healthy individuals at rest and task. The temporal features of brain states in epilepsy, such as FO, AR, are altered mainly at task. Interestingly, our results suggested the impaired brain state transition in epileptic patients, which can be explained by reduced entropy of TP. In addition, a radial basis function kernel (RBF)-based support vector machine (SVM) classification algorithm categorizes epileptic patients and healthy participants efficiently using FO and TP as multivariate features, demonstrating the robustness of FO and TP as biomarkers of brain state dynamics in Epilepsy.

4.1 Brain state dynamics differ between individuals with generalized epilepsy and healthy participants.

Distinct from most MEG studies focusing on the resting state of epilepsy, in the present study we would also like to explore the brain dynamics of epilepsy during a cognitive task. The brain is a complex system that constantly receives and processes information from the external world, and in many circumstances, there are clear triggers (the specific external stimuli) for seizures, so therefore we believe that task state studies may be more beneficial in improving our understanding of epilepsy. In addition, brain dynamics observed in the resting state may suffer from uncontrolled or unmeasured factors that could lead to misleading conclusions (Greene et al., 2023), in contrast task state investigations benefit from better control of variables and can yield more

convincing results.

A previous study demonstrated that there are no significant differences in SMN-related brain states between healthy controls and patients with generalized epilepsy in the resting state (Krzeminski et al., 2020), which is further supported by our results such as FO, DT and AR in the resting state. Notably, the FO of SMN-related brain states (state 6) is significantly decreased in the generalized epilepsy group during the Fibonacci counting task with eyes open. In addition, the FO of visual-related brain states (state 7) was significantly increased in generalized epilepsy group for the eyes open task. Considering that state 6 contains the posterior inferior parietal cortex (IPC), we speculate that visual top-down modulation is reduced in epileptic patients during the eyes-open Fibonacci task.

It has been demonstrated that cognitive impairment in patients with generalized epilepsy is associated with FPN dysfunction (Krzeminski et al., 2020) and increased cognitive demands may precipitate seizures (Yacubian et al., 2014). In our study, with increased cognitive demand from eyes-closed Fibonacci counting, FPN-associated brain state (state 3) dysfunction was observed in generalized epileptic patients, characterized by a significant increased AR in state 3. Interestingly there are no significant alterations in FO and DT at state 3 observed in epileptic patients during eyes-closed Fibonacci counting. We speculate that patients with generalized epilepsy are constrained to impaired cognitive functioning and to compensate for increased cognitive demands by elevating the AR. Boosting AR by fine-tuning FO and DT within a threshold range might be a minimally disturbing way to compensate the brain's cognitive demands.

In general, our results indicate that alterations in brain state dynamics in patients with generalized epilepsy mainly arise during task execution. Compared to the resting state, the study on brain states at task would probably be more effective in capturing the brain dynamics alterations in generalized

epileptic patients. Furthermore, our results indicate that patients with generalized epilepsy compensate for cognitive demands subject to visual input. Without visual input (closed-eye Fibonacci counting), cognitive demands are compensated by adjusting AR primarily. While with visual input (eyes-open Fibonacci counting), the cognitive demands are compensated by adjusting the FO instead. our findings provide evidential support for altered brain dynamics in generalized epilepsy at task.

4.2 Decreased entropy explains impaired brain state transition in generalized epilepsy patients.

Brain function depends on the ability to both access desired brain states and to rapidly transition between those states (He et al., 2022). Transition probabilities are used to characterize the likelihood of transitions between brain states and entropy quantifies the brain state complexity and transition flexibility to reflect brain function and its information processing capabilities (Greene et al., 2023, Keshmiri, 2020). Our results demonstrated significantly decreased entropy in generalized epilepsy group in states 5,6, and 8, indicating that individuals with generalized epilepsy suffer from reduced complexity and impaired ability to transition among these states. It has been proposed that neural signals with low complexity may contribute to the establishment of phase relationships between distributed neural populations, thereby enhancing the information exchange across functionally segregated regions (Zhen et al., 2024). Hence, the impairment of brain state transitions in generalized epilepsy might be associated with an abnormal synchronization resulting from elevated functional connectivity in widespread brain areas (Li Hegner et al., 2018). Moreover, it has been shown that brain state transitions are also affected by white matter connectivity (anatomical structure) and energy demands (Cornblath et al., 2020). A study on focal epilepsy (He et al., 2022) pointed out that individuals with focal epilepsy have significantly higher energy demands to achieve brain state transitions than healthy participants. We speculate that dysfunctional

energy metabolism could also be an important cause of brain state transition impairment in generalized epilepsy, however this needs to be confirmed by further studies.

Overall, limited transition of brain states in generalized epilepsy is associated with reduced entropy, suggesting that entropy may be a promising biomarker for quantifying brain function in generalized epilepsy.

4.3 Multivariate classifier based on FO and TP features efficiently discriminates healthy and epileptic individuals.

We validated the reliability of parameters characterizing brain states in explaining differences in brain dynamics between epileptic and healthy individuals using multivariate decoding with SVM classifiers. FO demonstrated favorable classification properties in both the independent condition and averaged cross-conditions. TP showed well-classified properties during averaged cross-conditions, resting state with eyes-closed (at rest) and Fibonacci counting with eyes-open (at task).

Altogether, our results demonstrate the potential of FO and TP as biomarkers for assessing altered generalized epileptic brain dynamics at rest and at task.

4.4 Limitations

There are some limitations in the present study. 1) In the present study, we assumed that brain activity at each time point was represented by a single brain state, which may not be able to reconstruct the actual brain dynamics. Brain states are highly associated with brain functions. The brain performs complex functions all the time, therefore different brain states that overlap in a spatial-temporal manner may be more consistent with the dynamic nature of the brain (Greene et al., 2023). Hence, the spatiotemporal evolution of brain state joint-expression needs to be emphasized in future studies. 2) The present study focuses on differences in brain state dynamics between generalized epilepsy

patients and healthy participants, and lacking evidence to the specific physiological functioning of each brain state and the correlation with epileptic symptomology. Brain state manipulation techniques are probably the key to solving this problem. Such manipulation refers to achieving given brain states (and consequent behaviors) in an invasive or non-invasive way to establish a causal relationship between the brain and the behavioral state (Greene et al., 2023). Such work can both elucidate the complex neurodynamic behind physiological and cognitive states and facilitate the application in clinical therapies of inducible brain states (Greene et al., 2023).

4.5 Conclusion

Our study indicates that the spatial co-activation-based description of brain activity (brain states) can effectively capture rapidly evolving brain dynamics. In epileptic patients, investigating brain dynamics allows for optimizing the diagnosis and treatment of patients with generalized epilepsy.

5 Summary

Epilepsy is a common neurological disorder affecting approximately 1% of the global population, leading to recurrent seizures and various neurological, cognitive, and social challenges. The pathophysiology of epilepsy is attributed to an imbalance between neuronal excitation and inhibition, prompting research to focus on the molecular and network-level mechanisms of epileptic seizures.

Recent clinical studies emphasize that epilepsy is a network disorder characterized by abnormalities in the intrinsic structure and dynamic organization of brain networks. Previous research on epilepsy networks has primarily focused on the stationary functional connectivity between brain regions. However, brain networks are inherently transient and dynamically adapting to changing external and internal environments. Stationary brain network analysis methods are insufficient to fully capture the nature of disruptions in epilepsy networks. In this dissertation, magnetoencephalography (MEG) is employed to collect high temporal resolution functional brain data. A novel analytical approach is used to capture transient brain state dynamics. Here, brain states are defined as distinct network patterns that are co-activated in space. Using this method, we compared the brain state dynamics between healthy controls and patients with generalized epilepsy at rest and task. Using machine learning algorithms, we identified nine common brain states shared by both epileptic patients and healthy individuals during rest and task performance. Temporal characteristics of brain states in epileptic patients, such as frequency occurrence (FO) and average duration (AR), mainly showed changes during tasks. Interestingly, our findings indicate that brain state transitions in epilepsy patients are impaired, as evidenced by a decrease in the entropy of transition probability (TP). Moreover, the support vector machine (SVM) classification algorithm with a radial basis function (RBF) kernel, utilizing FO and TP as multivariate features, effectively differentiated between epileptic patients and healthy participants, demonstrating the robustness of FO and TP as biomarkers

for brain state dynamics in epilepsy.

Our study suggests that statistical descriptions of changes of brain activity patterns can effectively capture rapid brain state dynamics. Differences in brain dynamics between generalized epilepsy patients and healthy individuals are more easily observed during arithmetic task. In summary, our research provides new insights into the brain dynamics of generalized epilepsy, which may contribute to optimizing the diagnosis and treatment of patients suffering from this condition.

Zusammenfassung

Epilepsie ist eine häufige neurologische Störung, die etwa 1 % der Weltbevölkerung betrifft und zu wiederkehrenden Anfällen sowie verschiedenen neurologischen, kognitiven und sozialen Beeinträchtigungen führt. Der Pathophysiologie der Epilepsie liegt ein Ungleichgewicht zwischen neuronaler Erregung und Hemmung zugrunde, was die aktuelle Forschung zur Diagnose und Behandlung von Epilepsien veranlasst hat, sich auf die molekularen und netzwerkbasierten neuronalen Mechanismen epileptischer Anfälle zu konzentrieren.

Jüngste klinische Studien betonen, dass Epilepsie eine Netzwerkerkrankung ist, die durch Anomalien in der intrinsischen Struktur und der dynamischen Organisation von Gehirnnetzwerken gekennzeichnet ist. Frühere Forschungen zu Epilepsienetzwerken haben sich hauptsächlich auf die stationäre funktionelle Konnektivität zwischen Gehirnregionen konzentriert. Allerdings sind Gehirnnetzwerke von Natur aus flüchtig und passen sich dynamisch an sich verändernde äußere und innere Umgebungen an. Stationäre Methoden zur Analyse von Gehirnnetzwerken reichen nicht aus, um die Art der Störungen in Epilepsienetzwerken vollständig zu erfassen. In dieser Dissertation wird die Methode der Magnetoenzephalographie (MEG) verwendet, um funktionelle

Gehirndaten mit hoher zeitlicher Auflösung zu erfassen. Ein neuartiger analytischer Ansatz wird genutzt, um transiente Dynamiken von Gehirnzuständen zu bestimmen. Hierbei werden Gehirnzustände als distinkte Aktivierungsmuster definiert. Mit dieser Methode wurde die Dynamik der Gehirnzustände zwischen gesunden Kontrollpersonen und Patienten mit generalisierter Epilepsie im Ruhezustand und während einer Arithmetikaufgabe verglichen. Mithilfe von maschinellen Lernalgorithmen identifizierten wir neun gemeinsame Gehirnzustände, die sowohl bei epileptischen Patienten als auch bei gesunden Personen während Ruhe und der Erledigung der Aufgaben auftreten. Temporale Merkmale der Gehirnzustände bei epileptischen Patienten, wie Häufigkeit des Auftretens (FO) und durchschnittliche Dauer (AR), zeigten hauptsächlich während der Rechenaufgabe Veränderungen. Interessanterweise weisen unsere Ergebnisse darauf hin, dass die Übergänge zwischen Gehirnzuständen bei Epilepsiepatienten beeinträchtigt sind, was sich in einer Verringerung der Entropie der Übergangswahrscheinlichkeiten (TP) niederschlägt. Darüber hinaus konnte ein auf einer Support-Vector-Machine-basierter (SVM) Klassifikationsalgorithmus, der Radiale Basis Funktionen (RBF) als Kernel verwendet, und der FO und TP als multivariate Merkmale nutzt, epileptische Patienten effektiv von gesunden Teilnehmern unterscheiden. Die Ergebnisse demonstrieren die Robustheit von FO und TP als Biomarker der Gehirnzustandsdynamik bei Epilepsie. Unsere Studie legt nahe, dass statistische Beschreibungen von Veränderungen der Gehirnaktivitätsmuster schnell auftretende Gehirnzustandsdynamiken effektiv erfassen können. Unterschiede in den Gehirndynamiken zwischen Patienten mit generalisierter Epilepsie und gesunden Personen sind während arithmetischer Aufgaben leichter zu beobachten. Zusammenfassend liefert unsere Forschungsergebnisse neue Einblicke in die Gehirndynamiken generalisierter Epilepsie, die zur Optimierung der Diagnose und Behandlung von Patienten mit dieser Erkrankung beitragen können.

6 Supplementary materials

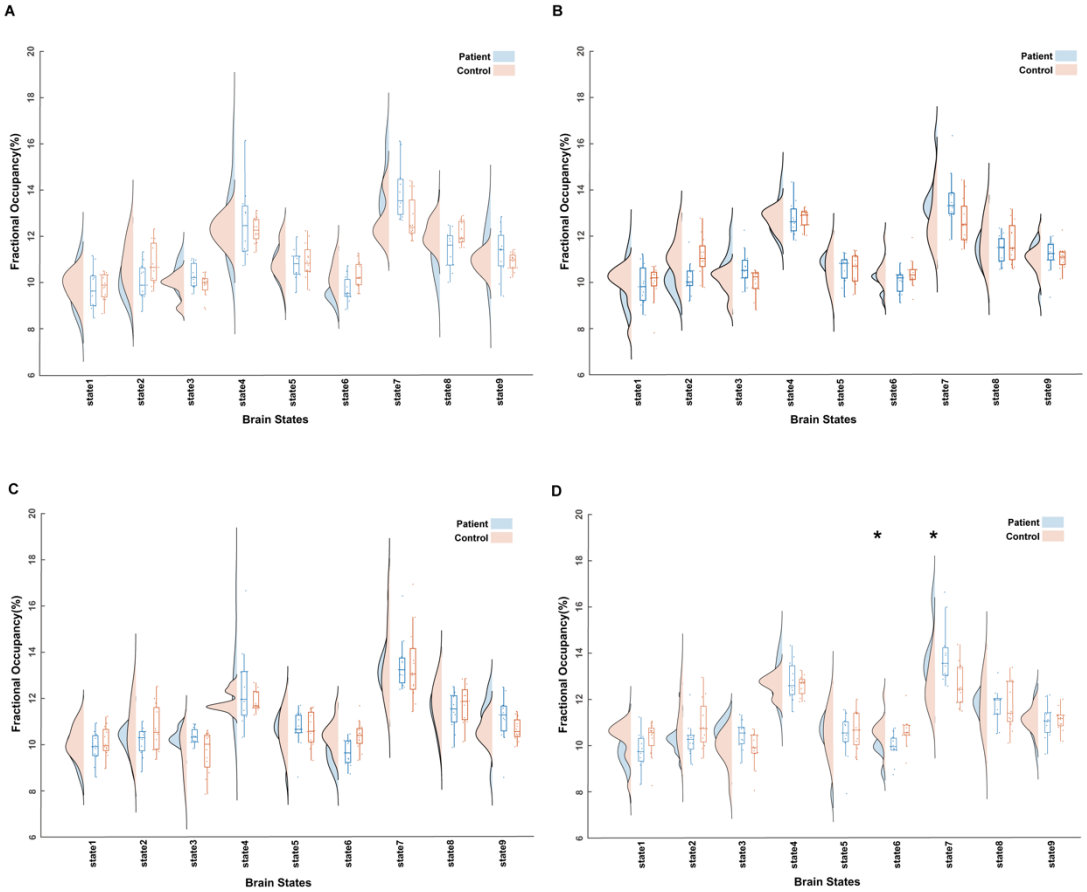

Fig.S1. Fractional occupancy in independent conditions between generalized epilepsy patients and healthy controls. A) Condition I, resting state eyes-closed. B) Condition II, resting state eyes-open. C) Condition III, Fibonacci counting with eyes-closed. D) Condition IV, Fibonacci counting with eyes-open. *p FDR <0.05 indicates that the P-value is less than 0.05 after multiple comparisons correction.

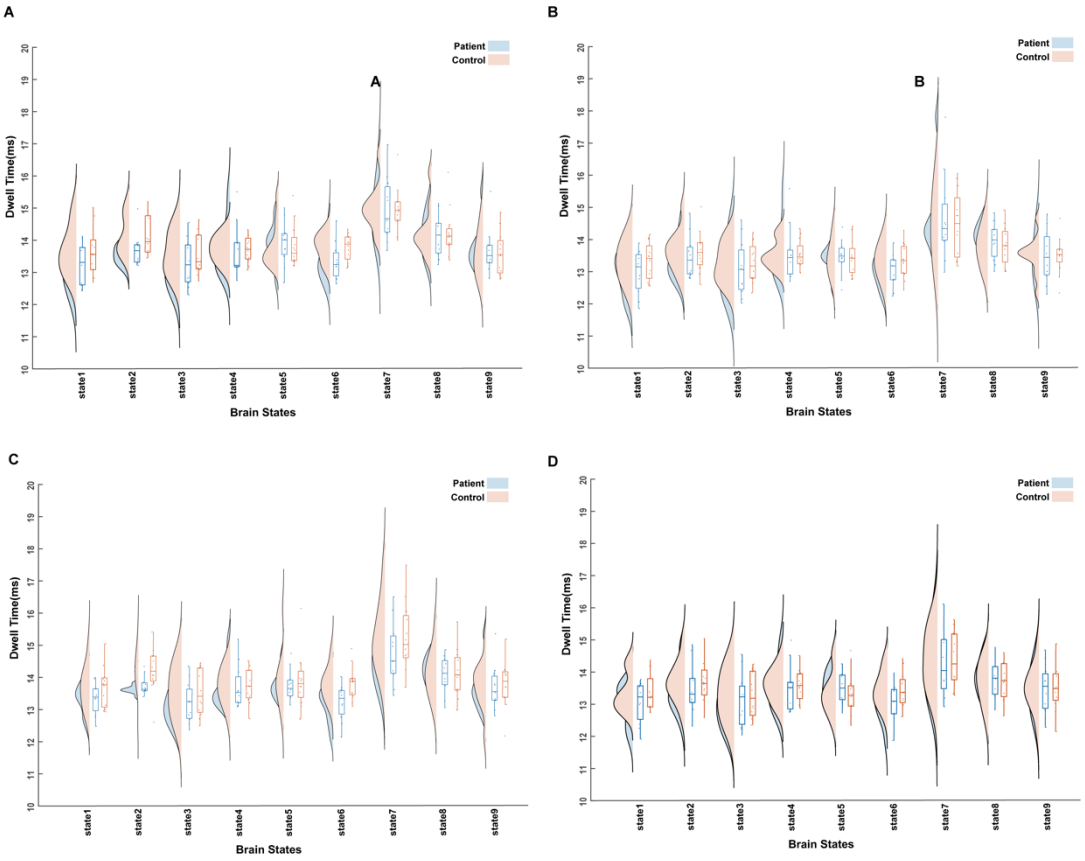

Fig.S2. Dwell Time in independent conditions between generalized epilepsy patients and healthy controls. A) Condition I, resting state eyes-closed. B) Condition II, resting state eyes-open. C) Condition III, Fibonacci counting with eyes-closed. D) Condition IV, Fibonacci counting with eyes-open. *p FDR <0.05 indicates that the P-value is less than 0.05 after multiple comparisons correction.

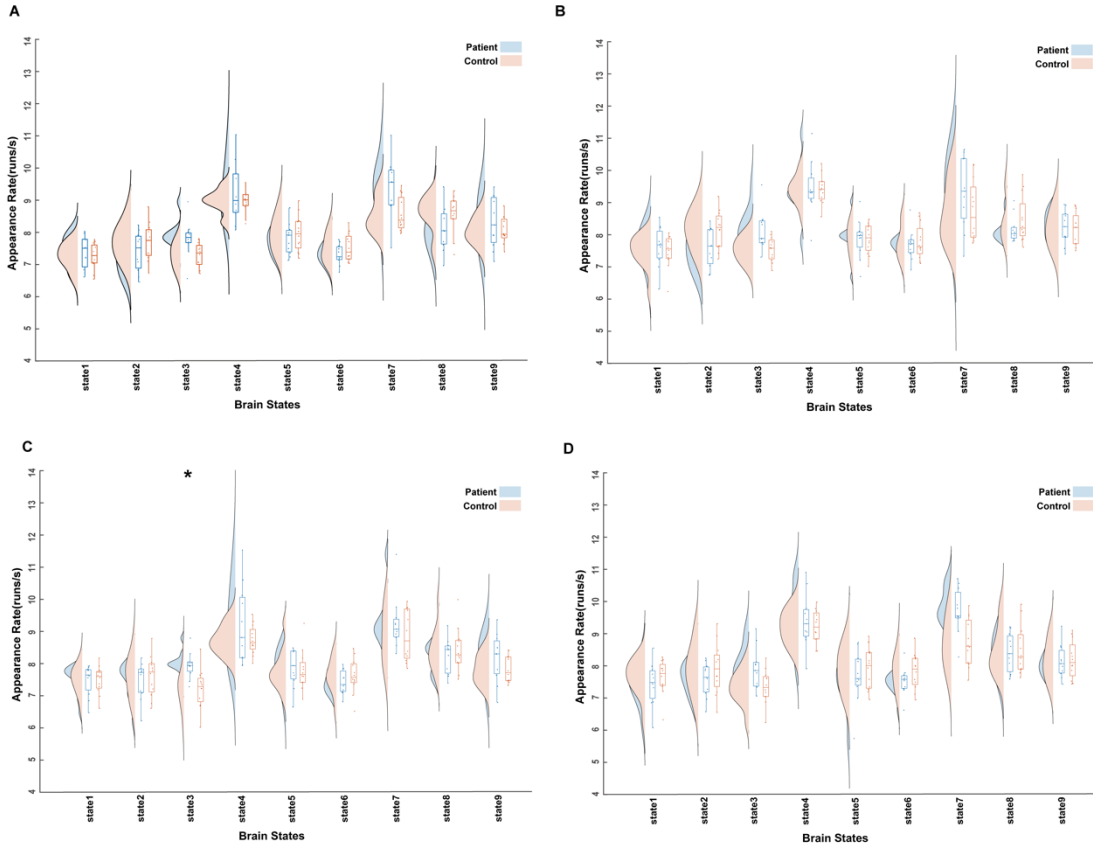

Fig.S3. Appearance Rate in independent conditions between generalized epilepsy patients and healthy controls. A) Condition I, resting state eyes-closed. B) Condition II, resting state eyes-open. C) Condition III, Fibonacci counting with eyes-closed. D) Condition IV, Fibonacci counting with eyes-open. *p FDR <0.05 indicates that the P-value is less than 0.05 after multiple comparisons correction.

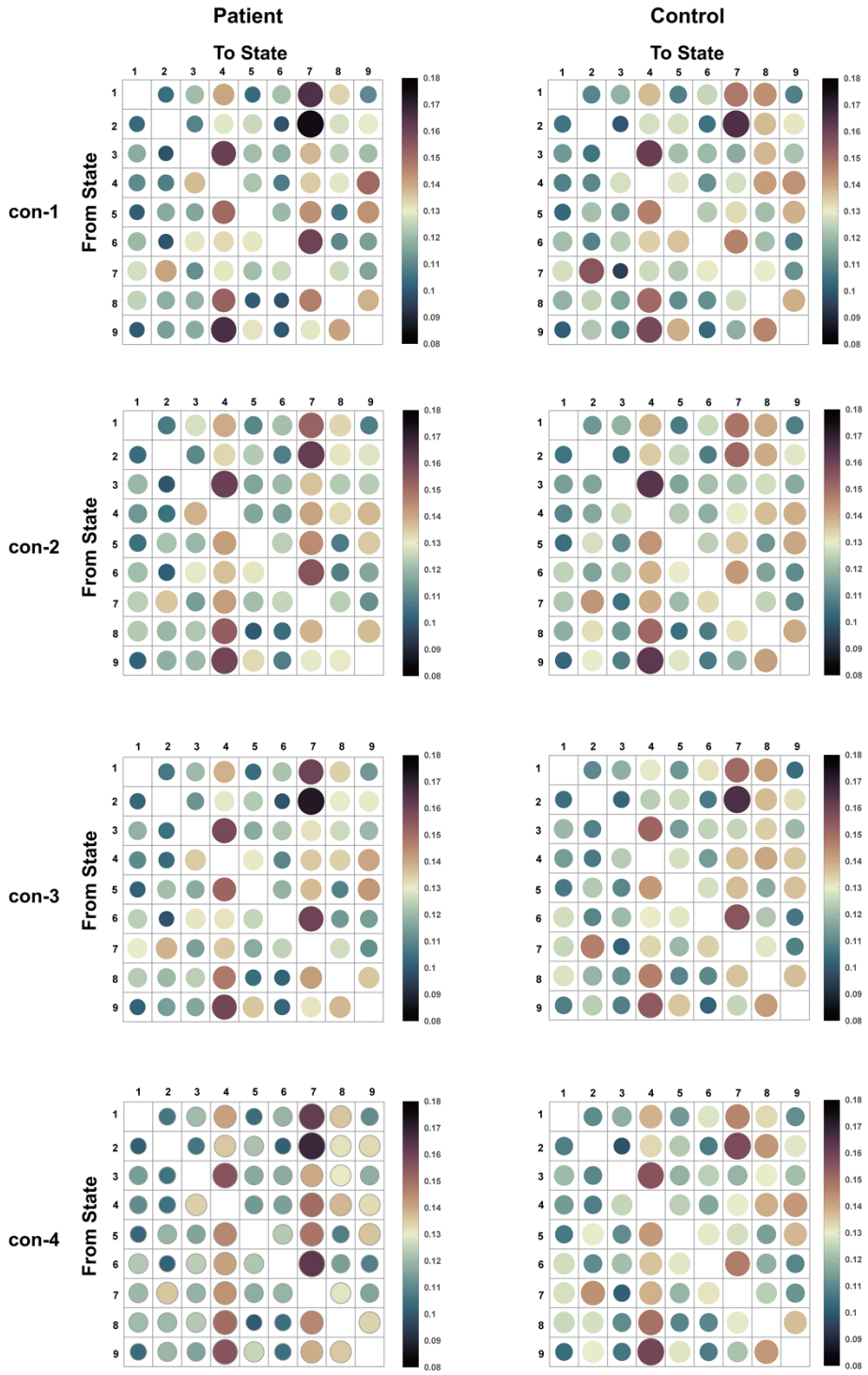

Fig.S4. Brain state transition matrix in independent conditions between generalized epilepsy patients and healthy controls. A) Condition I, resting state eyes-closed. B) Condition II, resting state eyes-open. C) Condition III, Fibonacci counting with eyes-closed. D) Condition IV, Fibonacci counting with eyes-open.

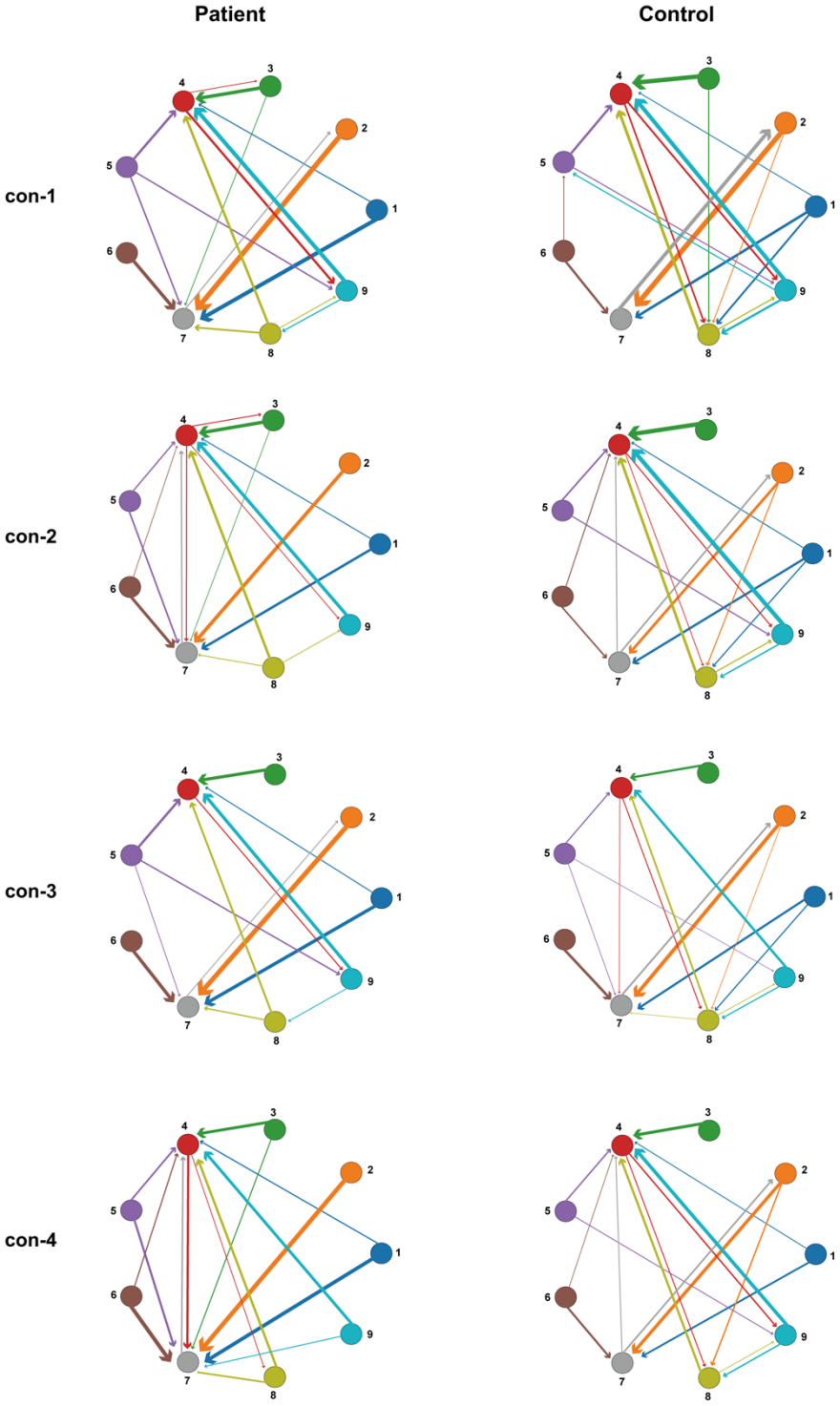

Fig.S5. Visualization of brain state transitions in generalized epilepsy patients and healthy controls in independent conditions. A) Condition I, resting state eyes-closed. B) Condition II, resting state eyes-open. C) Condition III, Fibonacci counting with eyes-closed. D) Condition IV, Fibonacci counting with eyes-open. Visualized with a threshold state transition probability of 13.6%, the thicker the line the higher the transition probability.

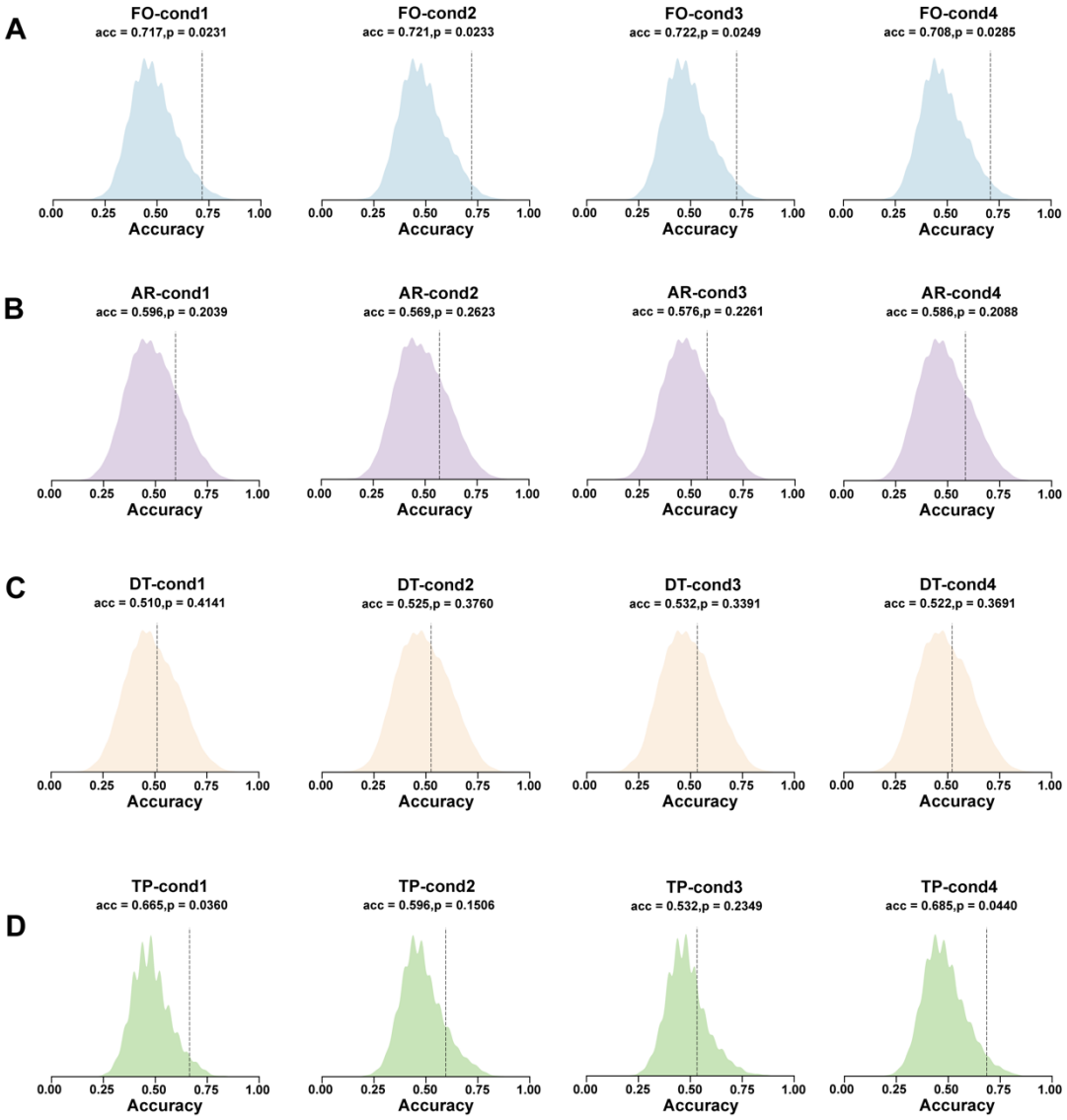

Fig.S6. Multivariate classification of generalized epilepsy patients and healthy controls under independent conditions. Classification accuracy of SVM classifiers using A) FO, B) AR, C) DT and D) TP as features in 4 conditions respectively. *p FDR <0.05 indicates that the P-value is less than 0.05 after multiple comparisons correction.

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Declaration of Contributions

This dissertation is the result of collaborative work involving several contributors, whose efforts I acknowledge here. The following declaration outlines the specific contributions made by me and others to the design, data collection, analysis, supervision, and writing of this work.

Study Design

The overall study design was conceptualized by myself, Hui Chen, in consultation with my supervisor, Prof. Dr. Christoph Braun. Prof. Dr. Braun provided guidance and critical feedback throughout the process, particularly during the initial stages of hypothesis formulation and experimental design. His expertise was essential in refining the study's conceptual framework.

Data Collection and Experiments

I personally carried out the majority of the experimental work presented in this dissertation, including:

1) All MEG recordings of epileptic patients (11) and healthy participants (12) at rest and task.

2) All MRI recordings of epileptic patients (11) and healthy participants (12). Some of the MRI collection (4 epileptic patients and 3 healthy participants) was assisted by PD. Dr Justus Marquetand, who helped with setting the MRI scanning paradigm and parameters.

In addition, the demographic data for epilepsy patients were provided by PD Dr. Justus Marquetand. I was responsible for organizing, conducting the statistical analysis, and presenting the results in Table 1, titled "Demographic Characteristics of Generalized Epilepsy Patients".

Data Analysis and Statistical Work

I performed the primary statistical analysis of the data, including:

1) MEG and MRI data processing and source analysis

2) Analysis of brain states and their dynamic features.

3) Statistical analysis.

4) Visualization of the results.

Dr. Antonino Greco assisted with the analytical script and statistical validation of Fig.10, Fig. 11 and Fig.S6.

Prof. Dr. Christoph Braun supervised the entire data processing and statistical analysis process.

Supervision

The research project was supervised by Prof. Dr. Christoph Braun and Dr. Antonino Greco. Prof. Dr. Braun offered academic guidance and technical oversight throughout all stages of the project, while Dr. Antonino Greco contributed significantly to data analysis, statistical processing, visualization, and interpretation.

Writing and Publication

I wrote the majority of the dissertation, including drafting and revising the manuscript. Prof. Dr. Christoph Braun provided editorial suggestions, helping to refine the text and improve the clarity of scientific arguments. Dr. Antonino Greco provided valuable suggestions for revising the methodology section, specifically regarding the “entropy calculation” and “multivariate classification”.

Acknowledgement of Other Contributions

I would like to acknowledge Mr. Jürgen Dax for his technical in equipment troubleshooting, which was essential to the completion of this work. I would also like to thank Mr. Sangyeob Baek and Mr. Davide Sometti for their insightful discussions and valuable experience sharing, particularly in MEG data preprocessing and source analysis.

In conclusion, while this dissertation is a collaborative effort, the majority of the intellectual and practical contributions, including study design, data collection, analysis, and writing, were made by me, with significant guidance from my supervisors and collaborators as mentioned above.

_____

Place / date / signature of doctoral candidate

Acknowledgements

As I look back on the past four years in Tübingen, I would like to express my heartfelt thanks to everyone who has offered me their help and support.

First and foremost, I am deeply grateful to my supervisor, Christoph Braun. Your patience and insightful guidance throughout my research have been invaluable. Without your support, I would not have had the opportunity to explore this fascinating field and complete this work.

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A very special thank you goes to my beloves girlfriend, Siyu. Your love, companionship, encouragement, and understanding have been a great source of strength. I appreciate all the moments we've shared exploring the world together.

Finally, I am deeply grateful for my family's unwavering love and support. Your constant encouragement and trust have provided me with the confidence to continually improve and become a better version of myself.