Imaging Network Alterations in Patients With Genetic Generalized Epilepsy and Their Healthy Siblings Using Magneto- and Electroencephalography

DISSERTATION

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Thesis Committee

Prof. Dr. Niels Focke
Clinic of Neurology, University Medical Center Göttingen

Prof. Dr. Alexander Gail
Sensorimotor Group, Cognitive Neuroscience Laboratory,
German Primate Center, Göttingen

PD Dr. Peter Dechent
Department of Cognitive Neurology, University Medical Center Göttingen

Members of the Examination Board

Referee: Prof. Dr. Niels Focke
Clinic of Neurology, University Medical Center Göttingen

2nd Referee: Prof. Dr. Alexander Gail
Sensorimotor Group, Cognitive Neuroscience Laboratory,
German Primate Center, Göttingen

Further members of the Examination Board

PD Dr. Peter Dechent
Department of Cognitive Neurology, University Medical Center Göttingen

Dr. Arezoo Pooresmaeili
Perception and Cognition Group, European Neuroscience Institute, Göttingen

Prof. Dr. Walter Paulus
Formerly Clinical Neurophysiology, University Medical Center Göttingen
Department of Neurology, Ludwig-Maximilians-University Munich,
Klinikum Großhadern

Prof. Dr. Michael Wibral
Department of Data-driven Analysis of Biological Networks, University of Göttingen

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Are we just our brains?
And are they more than the sum of their parts?

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Abstract

Genetic generalized epilepsy (GGE) is a common epilepsy syndrome and represents the largest group of epilepsies suspected to have a complex genetic etiology. Specific time windows for age of onset and various seizure types that rapidly engage bilateral networks of the brain characterize GGE. Another hallmark of GGE is the occurrence of brief, transient synchronized discharges in the 2-3 Hz range, as observed in the electroencephalogram. To date, there is no clear understanding of how large-scale brain networks behave in the absence of seizures or discharges, that is, during the interictal state in GGE. It is also unclear how this functional state relates to the genetic etiology of the disease. This dissertation presents three studies that address the interictal state in GGE and its functional underpinnings to support diagnostic and therapeutic innovations using electrophysiological imaging phenotypes:

In study I, patients with GGE and healthy individuals were measured during the resting-state using magnetoencephalography (MEG). Network power and phase-based connectivity were estimated following a whole-brain approach, and surface-based source analyses. An endophenotype approach was adopted, in which also the healthy siblings of the patients were studied to evaluate whether derived network alterations are genetically influenced.

In study II, recordings from high-density electroencephalography (HD-EEG) of the same study cohort were analyzed with similar methods as in the first study and statistically combined with the MEG results and structural measures to broaden the understanding of the functional imaging phenotype in GGE.

In study III, how power and connectivity vary across the lifespan was examined using a large-scale dataset from the Cambridge Centre for Ageing and Neuroscience (Cam-CAN) to advance knowledge of the biological meaning of these markers concerning normative lifespan trajectory and disease development.

Overall, this dissertation sheds new light on the interictal state and the causes and consequences of network alterations in GGE by revealing increased connectivity and power and evidence for a genetic contribution. The examinations of the study cohort using both techniques, HD-EEG and MEG, in a broad frequency spectrum adds significantly to the understanding of GGE to date and helps in comparing these findings with those of previous clinical studies. The work in this dissertation further promotes multimodal imaging in GGE that incorporates brain structural features in addition to electrophysiological markers. It also proposes to investigate behavioral and genetic correlates of power and phase-based connectivity across the lifespan and to investigate deviations from the norm that may lead to pathology in GGE. Eventually, this dissertation provides suggestions into how electrophysiological data might be linked to the genetic nature of GGE.

Contents

1 INTRODUCTION

1.1 EPILEPSY

The following sections provide the reader with an overview of the clinical presentation of epilepsy, the diagnostic process, causes of the disease, and treatment options. Further, the reader will be introduced to the concept of epilepsy as a network disease.

1.1.1 SEIZURES AND EPILEPSY

Today, epilepsy is conceptualized as a brain disease with a persisting disposition to epileptic seizures and its neurobiological, cognitive, and psychosocial consequences (Fisher et al., 2005). Individuals with an epileptic seizure present with a transient shift in behavior caused by abnormal excessive or synchronous neuronal activity (Fisher et al., 2005). Broadly summarized, motor and non-motor signs or symptoms are typical of seizures that may be accompanied by loss of consciousness. The severity and frequency of seizures vary greatly. Seizures may manifest as brief episodes of disturbed awareness, abnormal sensations, muscle twitches, or severe prolonged convulsions. Seizures may occur less than once a year or several times a day (Devinsky et al., 2018). About 10% of all individuals may experience an unprovoked seizure during their lifetime, but not all of them develop epilepsy (Bergey, 2016; WHO, 2019). In practice, the current clinical definition of epilepsy requires the occurrence of at least two unprovoked seizures with a minimum interval of 24 hours. A high recurrence risk after a first unprovoked seizure, that is, at least 60 % over the following ten years, can also suggest the diagnosis of epilepsy (Fisher et al., 2014). This broader definition, proposed by the International League Against Epilepsy (ILAE) allows clinicians to respond to specific circumstances, in which there is a high risk of recurrence in a patient such as after a stroke or trauma, or in cases of a well-defined epilepsy syndrome (Fisher et al., 2014). Provoked or acute symptomatic seizures are thought to be situational and differ mechanistically from chronic epilepsy with recurrent seizure activity (Shorvon & Guerrini, 2010). These seizures usually occur during or as consequence of a systemic insult, that lowers the seizure threshold in the brain, often due to toxins, metabolic factors, medication or acute illness such as infections (Bergey, 2016; Vezzani et al., 2016). The description of seizures, along with electrophysiological and imaging findings, is central to the diagnosis of the various types of epilepsy (Scheffer et al., 2017). Different causes for the occurrence of seizures are known and must be taken into account when making a diagnosis. Likewise, comorbidities such as learning disabilities and psychiatric disorders such as depression or autism spectrum disorders should be considered (Devinsky et al., 2018). Seizures can remit and epilepsy is considered resolved, if the person has been seizure-free for more than ten years and off-medication for more than five years, or has passed the age for an age-related epilepsy syndrome (Fisher et al., 2014).

1.1.2 EPIDEMIOLOGY

In total, around 50 million people worldwide are affected by epilepsy, making epilepsy one of the most common neurological conditions and a global burden (Beghi et al., 2019; Ngugi et al., 2010). This means that epilepsy is still a significant cause of premature mortality and residual disability, coupled with the notable economic impact due to loss of labor productivity and the need for medical care (WHO, 2019). The lifetime prevalence was estimated in a meta-analysis to be approximately 7.6 per 1,000 persons, and the annual cumulative incidence was estimated to be around 67.8 per 10,000 person-years (Fiest et al., 2017). Both, the prevalence and the incidence of epilepsy are much lower in high-income states than in developing or resource-poor countries, which account for up to 80% of all cases worldwide. This is probably due to endemic factors (Fiest et al., 2017) such as greater frequency of traffic accidents, infectious disorders, and birth complications (Singh & Trevick, 2016). In general, all age groups are affected, with the prevalence increasing with age, peaking in adolescence and early adulthood, and decreasing later in life (Fiest et al., 2017). The incidence rate tends to follow a U-shaped pattern with the highest incidences in infancy or early childhood and older age groups (Fiest et al., 2017), indicating the risk of developing the disease in these age ranges. According to the meta-analysis, both sexes seem to be affected in a similar way, although some studies report higher numbers for men (Fiest et al., 2017). This could be due in part to the fact that women are less likely to report the condition if they might be marginalized for it (WHO, 2019). In some places, epilepsy is still highly stigmatized and is a cause for restriction of human rights. For example, the right to education and marriage, or access to health and life insurance may be restricted, often preventing appropriate treatment and health care (WHO, 2019). Epilepsy can lead directly to death, unexpectedly ("sudden unexpected death in epilepsy") or from falls and burns, drowning, accidents, or status epilepticus. Comorbidities of seizures or adverse effects of medications (anticonvulsants or psychiatric drugs) such as obesity and cardiovascular effects or suicide can also be fatal (Devinsky et al., 2016). It is estimated that the mortality in affected individuals is 2.3 to 2.6 times higher than in individuals without epilepsy (Levira et al., 2017; Thurman et al., 2017).

1.1.3 DIAGNOSTIC PROCESS

Seizure as key symptom for classification

An accurate diagnosis and a classification system are critical for providing appropriate treatment and facilitating communication in clinical care and research. The ILAE provides an operational framework for the diagnosis of epilepsy and currently classifies epilepsy on three levels, namely seizure type, epilepsy type, and epilepsy syndrome (Scheffer et al., 2017). These guidelines are adapted to serve different clinical environments and resources available around the world. A diagnosis on all three levels is desirable, but not always feasible in the absence of lacking information or equipment. A thorough diagnostic workup should include neurological examination, routine EEG recordings, cranial imaging, and sometimes laboratory and genetic testing (Devinsky et al., 2018).

Differential diagnosis

Above all, differential diagnosis is a critical first step for the clinician to determine if the occurrence of an event is typical of a seizure. The clinical presentation of other disorders may mimic epileptic seizures and lead to misdiagnosis and inappropriate treatment (Devinsky et al., 2018). Imitators can be syncope and anoxic seizures, behavioral, psychological and psychiatric disorders, sleep-related conditions, paroxysmal movement disorders, migraine-associated disorders and others (a detailed list of imitators can be found elsewhere: https://www.epilepsydiagnosis.org/epilepsy-imitators.html). Differentiation from seizure mimics can be difficult because reports are often based, if at all available, on the statements of witnesses or the affected person. In addition, epilepsy may overlap clinically and mechanistically with other disorders, probably due to similar genetic and environmental factors (Winawer et al., 2013) causing co-occurrence and confounding.

Seizure classification

After ruling out non-epileptic events, seizures are classified as "focal", "generalized", or “unknown” depending on their initial manifestation. A focal seizure is considered to originate in the networks of one hemisphere, whereas a generalized seizure implies rapid involvement of bilateral brain networks. If the onset of the seizure is ambiguous or has been missed, it is classified as “unknown”. Focal seizures can be further grouped according to the level of awareness (and/or motor onset or non-motor onset symptoms). Another subcategory for all seizure types includes motor or non-motor onset symptoms shown at the earliest stage of the seizure (Scheffer et al., 2017).

Epilepsy types

When more information about the seizure event is available, such as findings from imaging and EEG, a higher-level diagnosis is indicated. Focal epilepsies comprise unifocal or multifocal events and the occurrence of unilateral seizures. EEG recordings during the interictal state, that is during resting-state, may reveal focal epileptiform discharges. A range of seizures can occur, including focal aware or impaired seizures, motor or non-motor seizures, and seizures with a focal onset pattern propagating bilaterally (“focal to bilateral tonic-clonic”). Generalized epilepsies usually involve absence, myoclonic, atonic, tonic, and tonic-clonic seizures including interictal generalized spike-wave discharges (GSWD) in the EEG. Less commonly, patients have both focal and generalized seizures and may be categorized as having "combined generalized and focal epilepsy". In other cases, the physician may not be able to determine the type of epilepsy because there is not enough information, or the seizure types are unclear. If there is sufficient evidence that the patient does have epilepsy, a diagnosis of unknown epilepsy type is made (Scheffer et al., 2017).

Epilepsy syndromes

Ideally, a cluster of clinical and imaging characteristics together constitute a distinctive disorder or syndrome (Scheffer et al., 2017). Age of onset or remission, seizure triggers, diurnal factors, specific comorbidities, such as cognitive or psychiatric dysfunction, or prognostic factors may be indicative of a syndrome. Known syndromes include Dravet syndrome, childhood absence epilepsy, West syndrome, and many others.

1.1.4 Etiology

Infectious, structural, metabolic, and immune etiology

In all stages of the diagnostic process, the etiology of the patient's epilepsy should be taken into account (Scheffer et al., 2017), although often the cause is unknown. In principle, any disturbances in the brain may transiently lower the threshold for seizure activity. A major risk factor for epilepsy are infections of the central nervous system (CNS), particularly in resource-poor countries (Vezzani et al., 2016). Examples include meningitis, encephalitis, neurocysticerosis, tuberculosis, HIV, cerebal malaria, and congenital infections (Devinsky et al., 2018). These infections may be associated with structural alterations in the brain. Structural abnormalities resulting in epilepsy can be also due to vascular events (e.g. stroke), traumatic brain injuries, brain tumors, hippocampal sclerosis, or be genetic such as cortical malformations (Sazgar & Young, 2019). A structural brain scan is usually part of the first investigations carried out to identify and assess the need for further treatment, for example by means of epilepsy surgery. Seizures can also be core symptoms of metabolic disorders with genetic defects or acquired chemical imbalances or immune disorders (Scheffer et al., 2017). In both cases, timely diagnosis is necessary to prevent cognitive and behavioral impairments in the course or to initiate specific therapeutic measures.

Genetic etiology

Epilepsy may also be genetic (Scheffer et al., 2017). This category refers to pathogenic variants or mutations that lead to epilepsy. Evidence of a genetic basis usually comes from a patient's family history, from family or twin studies or from investigations in clinical populations with the same syndrome. In the past few decades, gene sequencing methods have developed rapidly, leading to a high success rate in identifying genetic underpinnings of epilepsy (Thakran et al., 2020). This is mostly true for monogenetic forms of epilepsy, where a single gene or copy number variant are considered causative and almost entirely predictive of disease onset (Koeleman, 2018). Examples include heterogeneous forms of epilepsy with onset in infancy and childhood that are grouped under the term developmental and epileptic encephalopathies (Scheffer et al., 2017). Affected molecular pathways often involve ion channels, synaptic functions, and transcription factors (McTague et al., 2016). One of the most prominent epilepsy genes is *SCN1A*, a sodium channel subunit gene. Pathogenic *SCN1A*

variants can lead to a spectrum of disease phenotypes, such as mild forms like genetic epilepsy with febrile seizures plus (GEFS+) or severe forms like the well-known Dravet syndrome, in which up to 80% of patients carry such a variant (Devinsky et al., 2018; Mulley et al., 2005). Thus, the phenotypic heterogeneity underlying a single genetic cause requires careful evaluation of a genetic finding in the clinical context for outcome prediction and therapy (McTague et al., 2016). Conversely, multiple genes may be associated with an epilepsy syndrome, even in prototypical Dravet syndrome (Scheffer et al., 2017). Currently, genetic testing and diagnosis, when available, are successful in approximately 10-50% of epileptic encephalopathy cases (Allen et al., 2017; Carvill et al., 2013; Lemke et al., 2012; McTague et al., 2016). In fact, most common epilepsies such as generalized and non-acquired focal epilepsies presumably follow a complex inheritance pattern. This means disease susceptibility is attributed to multiple factors, implying that both complex genetic and non-genetic factors are important. Despite the clear clustering of genetic generalized epilepsy and focal epilepsy in families, only genome-or exome-wide association studies with large sample sizes have detected a few risk loci for common epilepsies to date (Helbig et al., 2009; May et al., 2018; Mefford et al., 2010; Striano et al., 2012; The International League Against Epilepsy, 2018), suggesting a heterogeneous genetic landscape and small effect sizes (Koeleman, 2018). It is notable that patients with genetic epilepsy in general do not necessarily have a family history of seizures or epilepsy. De novo mutations can occur in both severe encephalopathies or common epilepsies (Arsov, Mullen, Damiano, et al., 2012; Carvill et al., 2013; Claes et al., 2001) and the numbers are increasing (Scheffer et al., 2017). That means that new mutations may arise in an individual that may or may not be passed on to the next generation.
In summary, etiologic categories for epilepsy are diverse and not mutually exclusive. For example, a structural etiology may have its roots in genetic causes, both of which may be crucial for further treatment (Scheffer et al., 2017).

1.1.5 Molecular mechanisms

As mentioned above, the exact origin of the disease is not clear in many patients; however, epileptogenesis is thought to be due to genetic and epigenetic alterations, molecular and structural changes, including several cell types and levels in the brain (Devinsky et al., 2018). Animal models suggest widespread neuronal circuit dysfunction preceding seizure occurrence, leading to a state of hyperexcitability and a low threshold for the generation of seizures (Devinsky et al., 2018). After several decades of experiments *in vivo* on epileptic animals or *in vitro* by inducing seizure-like activity in brain sections, the notion of an imbalance between excitatory and inhibitory mechanisms inducing ictogenesis, that is, seizure initiation and progression, has become established (Staley, 2015). However, it is unclear, how this imbalance can lead to a persistent state of increased risk for spontaneous seizures, as it is the case in chronic epilepsies and thus in the vast majority of seizures (Staley, 2015). Another difficulty with this theory arises from the increasingly discovered bulk of genetic mutations associated with epilepsy. In addition to mutations that point to inhibitory or excitatory pathways, there are also those that directly alter neither (Ran et al., 2015; Staley, 2015). In

brief, several processes are likely to be involved in lowering the seizure threshold and making the brain susceptible to spontaneous seizures, including astro- and microgliosis, and neuronal plasticity accompanied by changes in gene expression and ion channel functions (Varvel et al., 2015). New discoveries in the area of molecular mechanisms will probably expand the view on the timing of seizures and the various etiologies, possibly including a diverse set of affected pathways (Staley, 2015), which is critical for therapeutic innovation.

1.1.6 Treatment options

Advances in the field of epileptogenesis are essential for therapeutic innovation. This is because the currently available treatment options do not alter the course of epilepsy, but only reduce the symptoms and the mortality (Ryvlin et al., 2011). After a thorough diagnostic procedure, tailored treatment can be initiated with the goal of minimizing seizures and comorbidities, thereby enhancing the quality of life in the patients (Pitkänen, 2010). The selection of antiepileptic drugs (AEDs) is based on the type of suspected epilepsy and the individual characteristics of the patient, such as age, life circumstances, use of other medications, as well as the pharmacokinetic profile and adverse effects of the AEDs (Glauser et al., 2013). Most of the available AEDs putatively target GABA and glutamate transmission, synaptic vesicle modulation, and / or block voltage-gated sodium or calcium channels (Devinsky et al., 2018). About half of the patients usually achieve seizure control with one AED, and another 13 percent using two AEDs. Very few patients benefit from a combination of more than two AEDs, and about 30 percent of patients do not respond even after trying different AEDs and therefore continue to experience seizures (Kwan & Brodie, 2000). In some of these cases, resective surgery of epileptogenic tissue can lead to long-term seizure control depending on how well the epileptogenic zone can be mapped (Devinsky et al., 2018). This procedure requires long-term EEG video monitoring and structural magnetic resonance imaging (MRI), preferably also positron emission tomography (PET) and magnetoencephalography (MEG) (O'Brien et al., 2008; Rampp et al., 2019). Many of the patients who have non-lesional drug-resistant focal epilepsy also have intracranial electrodes implanted in order to more precisely track the seizure origin, plan surgery, and avoid loss of critical brain function. Alternative therapies have been shown to be effective in some patients, such as vagus nerve stimulation or dietary therapies (Devinsky et al., 2018).

1.1.7 Understanding and addressing epilepsy from a network perspective

Invasive electrophysiology in patients has contributed remarkably to new insights into seizure initiation, progression, and seizure self-termination. Recordings of electrocorticograms at the brain surface or stereotactic EEG recordings in deep brain regions are usually performed in drug-resistant epilepsy patients (Kramer & Cash, 2012). Although intracranial recordings can provide excellent insight into ictogenesis in focal epilepsies, the signal strength at the electrodes decreases rapidly with distance, and the sampling zone in the brain is sparse (Parvizi & Kastner, 2018). Also, in generalized seizures, invasive procedures are usually not applicable because extended bilateral networks are already affected at the onset of the

seizure. Therefore, whole-brain mapping using non-invasive methods are essential pieces of the puzzle to uncover global network mechanisms in generalized as well as focal seizure types. EEG and MRI are typically used clinically, but diffusion and functional MRI (dMRI and fMRI), MEG, computed tomography, PET, and spectroscopy are also commonly used techniques. Notable advancements in hardware, methods, analysis, and application of neuroimaging techniques have furthered the field, resulting in improved presurgical evaluations and led to the conceptualization of epilepsy as a network disorder (Goodman & Szaflarski, 2021). Ultimately, changes at the molecular level are thought to lead to structural and functional neuronal connections at the systems level, resulting in epileptic activity. The development of generalized and focal seizures is assumed to rely on various brain networks and thus distal and distributed perturbations (Devinsky et al., 2018), which can be studied by applying brain network models (Goodman & Szaflarski, 2021). Understanding epilepsy from a network perspective thus represents a shift toward an integrative view of brain structural and functional alterations that encompasses multiple spatial and temporal scales (Bassett & Sporns, 2017). This includes the study of comorbidities of epilepsy, such as cognitive or behavioral problems, imaging of patients' brain activity and connectivity while performing tasks and during rest. In particular, the resting-state framework has found wide application in pathological conditions to develop biological meaningful markers of disease (Fox & Raichle, 2007). Spontaneous resting-state brain activity has been found to be temporally correlated across different regions when a study participant is asked to sit or lie still with eyes open or closed in the absence of a task or external stimuli. This observation was initially made in the 1990s for the somatosensory network (Biswal et al., 1995) and then for the default mode network (DMN), which comprises the medial prefrontal cortex, posterior cingulate cortex, precuneus, and parietal regions (Greicius et al., 2003; Raichle et al., 2001). Later, other neurocognitive networks were described, including regions from sensory to higher order control systems (Damoiseaux et al., 2006; De Luca et al., 2006; Fox et al., 2005). This intrinsic network organization, as determined by fMRI, is very stable (Gratton 2018), relatively independent of external tasks (Cole et al., 2014; Krienen et al., 2014), and under genetic control (Glahn et al., 2010). Suspensions in resting-state networks have been demonstrated for various epilepsy types, particularly in the DMN (Yang et al., 2021). In contrast, although electrophysiology has a long tradition in the clinical setting and in epilepsy research, quantitative resting-state analyses are less common but are further discussed in this work for genetic generalized epilepsy as a way to study network dysfunction. The conceptualization of whole-brain functional connectivity networks based on neurophysiological signals is rather modern, despite early views of long-range communication in the brain through oscillatory brain activity and synchronization (Sadaghiani & Wirsich, 2020). The reasons certainly lie in methodological challenges, such as how such a network looks like at different frequencies, or on which signal properties it should be constructed (Sadaghiani & Wirsich, 2020). In addition, the application of graph theoretical models on structural and functional brain networks (Bassett & Bullmore, 2006; Rubinov & Sporns, 2010; Sporns et al., 2005) has led to the investigation of brain topology in epilepsy, thereby identifying disruptions in, for example, network efficiency and integration.

Overall, the characterization of network dysfunction in epilepsy at multiple levels has led to new hypotheses and is associated with the hope of achieving a holistic and integrative understanding of the disease in the future and moving toward biologically informed diagnosis and treatment.

1.2 Genetic Generalized Epilepsy

In the following, the reader will become familiar with a specific epilepsy syndrome, the genetic generalized epilepsy (GGE), or, as previously called idiopathic generalized epilepsy (IGE), the study of which is the focus of this dissertation. GGE makes up 15-20% of all epilepsies and is the largest group of genetic epilepsies (Jallon & Latour, 2005). In addition to clinical and etiological aspects, the current status of functional and structural brain changes in GGE will be discussed.

1.2.1 Clinical presentation

Patients with GGE present with typical epileptiform EEG-features and with one or several of three common seizure types such as absence seizures, myoclonic and tonic-clonic seizures. Absences occur with abrupt loss of awareness and brief bursts of spike and wave (Mullen et al., 2018). Patients usually do not respond to external stimuli for a few seconds and show motor arrest (Panayiotopoulos et al., 1989). In contrast, myoclonic seizures are jerks involving axial or upper limbic muscles usually without impairment of awareness and are observed with spike or poly-spike wave discharges in EEG recordings. These motor seizures can evolve into tonic-clonic seizures (formerly called grand mal) lasting around one to three minutes with loss of consciousness and rhythmic bilateral muscle contractions (Vorderwülbecke et al., 2021). Most often, all three seizure types occur during the wake state, but subtle absences or jerks are observed during sleep (Vorderwülbecke et al., 2021). Myoclonic and tonic-clonic seizures typically occur in the first two hours after awakening and can be triggered by alcohol consumption or sleep deprivation the night before (Janz, 2000; Panayiotopoulos et al., 1994). Absences can be induced by hyperventilation (Panayiotopoulos et al., 1989). Other provoking factors for all seizure types can be visual stimuli such as flickering lights in a natural setting or strobe lighting, specific geometric patterns as well as complex cognitive and motor tasks (Ferlazzo et al., 2005).

Based on the clinical presentation and age at onset, GGEs are classified into four subtypes. Childhood absence epilepsy (CAE) typically begins between the age of four and eight years and is characterized by multiple absences during a day (Mullen et al., 2018). In juvenile absence epilepsy (JAE), the seizure frequency is lower, and the onset is between the age of 12 and 16 years of age (Vorderwülbecke et al., 2021). The criteria for juvenile myoclonic epilepsy (JME) include myoclonic seizures but do not exclude the occurrence of absences and tonic-clonic seizures and usually have onset between the age of 10 and 25 years (Vorderwülbecke et al., 2021). Patients with only generalized tonic-clonic seizures are assigned to a separate category (GGE-GTCS) that has a broader window for age at onset, ranging from childhood to the fourth decade of life and peaking at 16-18 years of age (Vorderwülbecke et al., 2017). These definitions of the subtypes though widely accepted and helpful in clinical practice are largely descriptive (Scheffer et al., 2017). In about 20% of patients with GGE (Vorderwülbecke et al., 2021), the clinical presentation cannot be clearly assigned to one of the four subtypes. Also, from a genetic point of view, the boarders between the subtypes seem to be vague, as

more than one type occurs in families with GGE cases (Marini et al., 2004) and shared genetic risk factors have been found (The International League Against Epilepsy, 2018). This argues for a neurobiological overlap of the subtypes rather than clear lines between them. In addition, long-term seizure and psychosocial outcome in adult-onset GGE have been highly similar across the subtypes (Vorderwülbecke et al., 2017). Also, mild cognitive impairment has been reported for GGE (see also Other Contributions), and possible differences and similarities between GGE subtypes are the subject of ongoing research (Ratcliffe et al., 2020).

1.2.2 Etiology of GGE

Several independent twin studies starting in the 1950s have provided strong evidence for major genetic contribution to GGE (Lennox, 1951). Early casewise concordance rate estimates that yielded values around 0.76 in monozygotic twins (MZ) versus 0.33 in dizygotic twins (DZ) (Berkovic 1998) have been confirmed in subsequent studies (Vadlamudi et al., 2004; Vadlamudi et al., 2014). A casewise concordance rate reflects the probability that one member is affected given that the other is affected (McGue, 1992). This rate was higher for GGE than for focal epilepsies (0.36 versus 0.05) or for seizure occurrence in general (0.62 versus 0.18) (Berkovic et al., 1998). MZ twins are largely genetically identical, whereas DZ twins share approximately 50% of the inherited genetic variation. A comparison of the two types of twins is thought to cancel out environmental influences, leaving the difference for the genetic contribution to phenotypic variability. Despite these early reports on the genetic contribution to GGE, gene discovery has been challenging up to date (The International League Against Epilepsy, 2018) as most GGEs presumably follow complex inheritance (Section 1.1.4), and thus, rare variants and many common variants are likely involved in the cause of GGE (Koeleman, 2018; Vadlamudi et al., 2014). Nevertheless, the history of genetic variant detection in GGE will be briefly outlined below.

As with other diseases following complex inheritance, variants in single genes have been identified in GGE (Mullen et al., 2018). In families with autosomal dominantly inherited GGE, variants in the *GABRG2* and *GABRA1* genes encoding subunits of one type of the gamma-aminobutyric acid receptor ($GABA_A$) were found to be causative (Wallace et al., 2001). Also, loss of function in the *SLC2A1* gene, which encodes the glucose transporter GLUT1, is observed in approximately 1% of individuals with GGE and in 10% of those with typical absence seizures that begin before the age of four years (Arsov, Mullen, Damiano, et al., 2012; Arsov, Mullen, Rogers, et al., 2012; Suls et al., 2009). GLUT1 is an important glucose transporter at the blood-brain barrier (Mullen et al., 2018), and its dysfunction is associated with movement disorders and absence seizures (Mullen et al., 2010; Weber et al., 2008) as well as severe metabolic encephalopathies and intellectual disability (De Vivo et al., 1991). Other linkage and candidate gene studies produced conflicting results or were not able to confirm the findings, probably due to insufficient statistical power (Koeleman, 2018).

After a long period of stagnation in gene discovery, the first genome-wide association study (GWAS) in 2009 shed new light on the field (Helbig et al., 2009). GWAS are conceptually designed for a “common disease common variants” hypothesis and enables a genome-wide

structural assessment of single nucleotide polymorphism (SNP) alleles. With this new technology deletions of chromosomal segments (also copy number variants (CNV)) at chromosomes 15 and 16, were detected in GGE (de Kovel et al., 2010; Helbig et al., 2009). Remarkably, these microdeletions had earlier been related to a spectrum of neurodevelopmental disorders (Miller et al., 2009), including autism, schizophrenia (Stefansson et al., 2008), and mental retardation (Sharp et al., 2008), but also occur in rare instances in unaffected individuals, suggesting that these CNVs should be considered risk factors rather than disease causation. Overall, these findings established a new perspective on the genetic architecture of GGE, underpinning the complexity with rare genetic variants.
In the following it became clear, that large sample sizes in the order of several ten thousand individuals with extended international collaboration are required to attain sufficient power for the detection of genetic risk in common epilepsies such as GGE (Koeleman, 2018). The formation of a meta-collaboration, the ILAE consortium on complex epilepsies, yielded the largest epilepsy GWAS to date, comprising 15,212 common epilepsy cases and 29,677 controls in total (The International League Against Epilepsy, 2018). Sixteen risk loci have been detected, of which 11 were new and the others previously reported. The majority of these could be attributed to GGE or GGE subtypes, compatible with the notion of higher heritability estimates for GGE than focal epilepsies. Enrichment analyses indicated that the observed risk variants are involved in the regulation of gene expression in the brain, particularly in the dorsolateral prefrontal cortex (The International League Against Epilepsy, 2018). The authors further pinpointed about 21 genes (for all common epilepsies), including known epilepsy genes related to ion-channel function (e.g. *SCN1A*), transcription factors, and vitamin B_6 metabolism. Moreover, genetic overlap among the GGE subtypes and between common and rare epilepsies had been noted (The International League Against Epilepsy, 2018). Nevertheless, the sample size of this mega-analysis was still modest compared with similar approaches in other common neurological disorders (The International League Against Epilepsy, 2018). Besides common variants, ultra-rare variants have also been identified using whole-exome sequencing, specifically a gene set encoding $GABA_A$ receptors in individuals with GGE. (May et al., 2018).
In summary, over the years and with the innovations in the field of genomics, some considerable insights have been gained into the complex genetic etiology of GGE, but further approaches and advances are needed to find additional variants and to develop a better understanding of the functional impact of genetic risk factors.

1.2.3 Treatment and outcome

The diagnosis of GGE is made on clinical grounds, as described in Section 1.1.3, and on the basis of short-term EEG recordings and sometimes in an overnight recording. Additional genetic testing or imaging studies may be necessary for the differential diagnosis. Antiepileptic medication tailored to the specific GGE type, as well as sex and comorbidities is the primary therapy once the diagnosis has been confirmed. Similar to AEDs used in other epilepsies, the putative mechanisms of the AEDs used in GGE include blockade of voltage-gated sodium and

calcium channels, enhancement of GABAergic transmission, inhibition of glutamatergic transmission, or synaptic vesicle modulation (Devinsky et al., 2018). Besides pharmacological treatment, training in self-management of the disease, avoidance of seizure triggers, education and psychological intervention are essential. Nevertheless, the disorder is drug-resistant in about 12-36 % of the patients with GGE in adulthood, and they experience seizures despite adequate treatment (Cerulli Irelli et al., 2020; Kwan & Brodie, 2000; Mohanraj & Brodie, 2007). This is particularly the case when more than one seizure type is present, the seizure onset is in childhood, a status epilepticus had occurred, or when psychiatric comorbidities are present (Gesche et al., 2020; Gesche et al., 2017). Nevertheless, the long-term outcomes in GGE are generally more favorable than in many other forms of epilepsy (Alsfouk et al., 2019; Beghi et al., 2019).

1.2.4 Functional network dynamics in GGE

As previously described, patients with GGE can experience absence, myoclonic and/or tonic-clonic seizures. By convention, these seizures are thought to engage the cortex in a symmetrical way, but about one third of the patients also show focal semiology (Usui et al., 2005) or exhibit focal or asymmetric ictal EEG features (Leutmezer et al., 2002). GGE is typically characterized by short transient episodes of synchronous EEG discharges occurring bilaterally, but often with an anterior predominance (Leutmezer et al., 2002). These GSWD can be detected using EEG during the interictal, that is the non-seizing period, and commonly have a frequency around 2-3 Hz (see **Figure 1** for an example) (Sazgar & Young, 2019). The role of GSWD towards epileptic network dynamics is not entirely clear. They could be a precursor or a driver of seizure activity or even protect against seizures (Chang et al., 2018). Many studies have focused on the localization of GSWD to map the origin of activation, but also to investigate the wide network implications before and after these events. There is strong evidence from multiple imaging modalities that thalamocortical and cortico-cortical networks play a central role in GGE (Bernhardt et al., 2009; Blumenfeld et al., 2003; Larivière et al., 2020; Moeller et al., 2008). Studies in animal models also suggest that thalamo-cortical circuits are involved in seizures (Maheshwari & Noebels, 2014), but also that the somatosensory cortex may play a role in the initiation of GSWD (Sitnikova & Van Luijtelaar, 2007). In humans, network behavior around GSWD has been altered for an extended period, suggesting high sensorimotor network synchrony along with low posterior synchrony before GSWSD onset and involvement of frontal, parietal, and occipital regions during GSWD (Tangwiriyasakul et al., 2018). This raises the question of how network changes in GGE behave in the phases without epileptiform activity, that is, interictal, from which GSWD and seizures may eventually evolve. Studies that have examined patients with GGE using fMRI during the resting-state have consistently demonstrated connectivity changes in the default mode network (DMN) (Parsons et al., 2020), which were common to all GGE subtypes but also to other epilepsies (Yang et al., 2021). It has been suggested that there is a higher likelihood for dynamic state transitions in the DMN of patients with epilepsy than of controls, potentially reflecting effects of a distorted excitation-inhibition balance in epilepsy (Yang et al., 2021). GGE-typical fMRI changes (Gonen

et al., 2020; Yang et al., 2021) also affected other regions, such as frontal (McGill et al., 2012), cingulate (McGill et al., 2012) and cerebellar (Kay et al., 2014) areas. EEG/MEG studies providing regional, that is, source-related, information for resting-state alterations in GGE are less numerous and will be discussed later in this work (Chapters 4.1 and 4.2). In general, EEG and MEG can greatly complement findings derived by functional MRI with a much greater temporal precision, are less sensitive to vascular confounds, and measure neuronal activity more directly than fMRI (Da Silva, 2013). EEG/MEG studies have indicated a less optimized brain network organization, with a tendency towards stronger local clustering and integration (Chavez et al., 2010; Chowdhury, Woldman, et al., 2014; Elshahabi et al., 2015). Evidence from the fMRI literature has been rather conflicting, pointing to methodological inconsistencies for graph theoretical network analyses across modalities (Pegg et al., 2020). It can be said that in addition to the occurrence of GSWD and seizures, resting-state and topological network changes were also found in GGE, but to gain further insight into the nature of GGE, these need to be further investigated, especially with higher temporal resolution.

1.2.5 Structural architecture in GGE

MRI scans in patients with GGE usually appear normal on clinical inspection (Woermann et al., 1998), but in the last few decades quantitative MRI analyses have revealed subtle structural alterations. In specific, cortical thickness reductions in bilateral precentral gyri and reduced volume of the thalamus have been most prominent (Bernhardt et al., 2009; Larivière et al., 2020; Whelan et al., 2018). Interestingly, these particularly subcortical grey matter atrophies were related to structural and marginally, to functional subcortico-cortico hubs, that is to highly connected regions, and to fronto-central connectivity profiles. These findings were distinct from those in temporal lobe epilepsies, pointing to specific effects for GGE (Larivière et al., 2020). Microstructural brain alterations have also been detected in fiber pathways, and although modest compared with focal epilepsies (Hatton et al., 2020), they support the hypothesis of a fronto-thalamic involvement in GGE (Deppe et al., 2008; Focke et al., 2014; Keller et al., 2011). Further, commissural, projection and corticocortical association pathways seem to be affected in GGE (Hatton et al., 2020), as observed using diffusion MRI metrics reflecting fiber density, axonal diameter, and myelination in white matter (Beaulieu, 2002). Graph theoretical analyses of structural data point to a more random network topology, which may indicate a loss of network efficiency and is also likely to affect brain functional architecture in GGE (Pegg et al., 2020). While structural alterations in focal epilepsies have often been considered as progressive, thus changing with duration of the disease (Caciagli et al., 2017), this association is less clear for GGE (Bernhardt et al., 2009; Hatton et al., 2020; Larivière et al., 2020; Wandschneider et al., 2019; Whelan et al., 2018). In both cases, the evidence is based on cross-sectional designs limiting the interpretation. Moreover, disentangling long-term disease effects from aging or medication effects and genetic predisposition has been difficult and requires longitudinal studies (Caciagli et al., 2017).

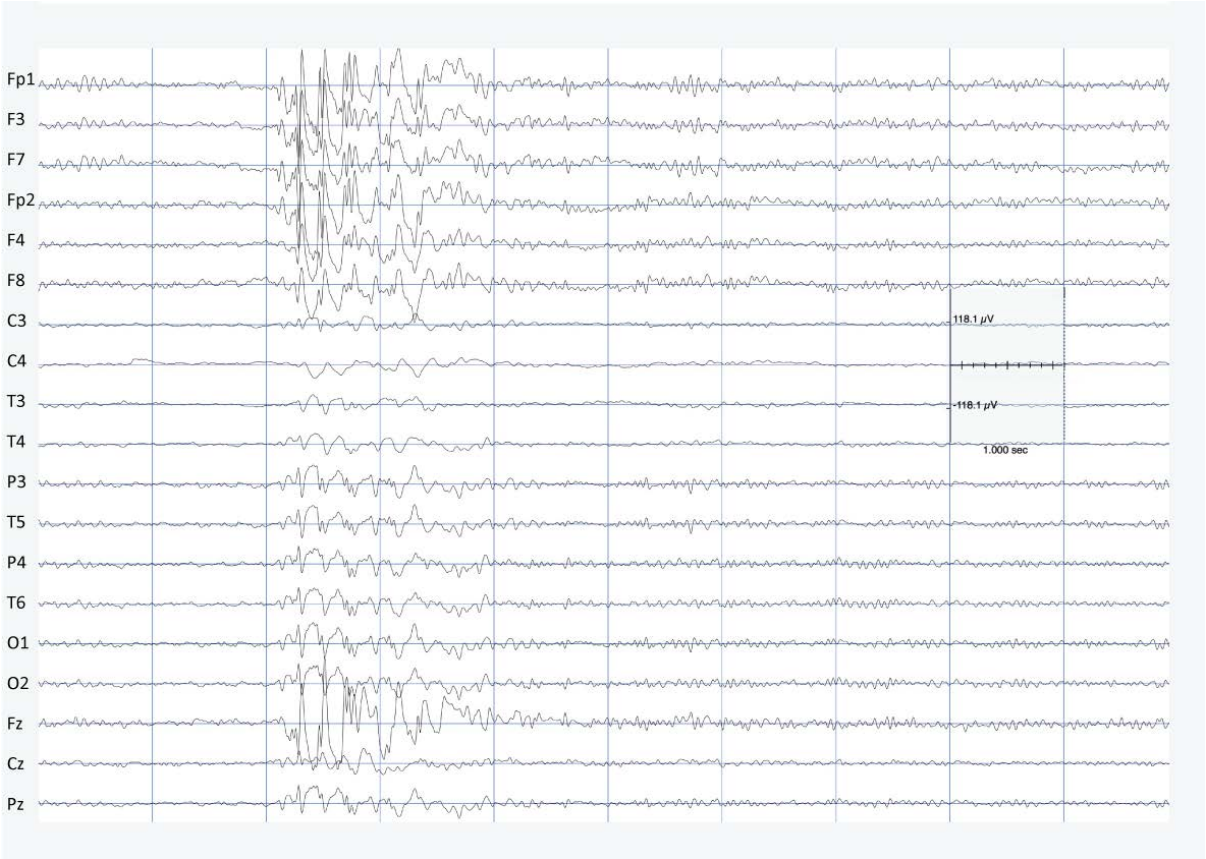

Figure 1 | Example of generalized spike-wave complexes of ~ 3 Hz

Shown are 10 seconds of a HD-EEG recording during resting-state eyes-closed (10-20 montage, microvolts). Patient is a 36-year-old with juvenile absence epilepsy (age at onset: 16 years). Note that the spike-wave discharges have frontal dominance (as observed in the frontal electrodes). Odd numbers represent the electrodes on the left hemisphere. Even numbers represent the electrodes on the right hemisphere. C = central, F = frontal, O = occipital, T = temporal, z = midline.

1.3 From Imaging to Genetics in GGE

One of the pieces of the puzzle to be uncovered that could advance the understanding, diagnosis and treatment of GGE is its genetic underpinnings. This chapter will present ways in which brain imaging can serve this purpose, as well as previous efforts in this direction.

1.3.1 Bridging the gap using genetically influenced imaging phenotypes

Imaging methods are used in an attempt to trace the neurobiological basis of GGE and over the last decades, advances in imaging research have led to a better understanding of the epileptic network. However, how the genetic make-up of GGE leads to the manifestation of symptoms and large-scale brain network alterations is still vague. To bridge the gap between imaging findings in GGE and the complex genetic basis, we have adopted an endophenotypic study approach, which will be introduced here. Endophenotypes are measurable quantities that are related to an illness and presumably genetically simpler than the genetics of a complex disorder itself (Gottesman & Gould, 2003). The idea is to advance gene discovery and mechanisms in diseases for which a number of risk loci have been identified, but which can explain only a small fraction of genetic variance. It is assumed that a smaller sample size than in large-scale genetic studies is required to detect the genetic architecture of endophenotypes, as a lower polygenicity and stronger effect sizes are expected than for the "full" clinical disorder in question (Glahn et al., 2014). Endophenotype research was introduced into psychiatry research in 1987 by Irving Gottesman and colleagues and has been resonating strongly ever since in the field (Roffman, 2019). The reason for this lies in the phenomenological or symptom-based and uncertain conceptualization of psychiatric disorders, which promoted efforts towards a biologically oriented classification. The term endophenotype was first described by John and Lewis in 1966 in studies of grasshoppers and referred to the "internal, microscopic condition" (endophenotype) that provided more information about their geographic distribution than their (external) appearance (John & Lewis, 1966). The hope in complex diseases is that clinical phenotypes can be better linked to underlying molecular biological causes and that the clinical overlap of diagnostic categories can be better explained. Endophenotypes can be of any behavioral or physiological nature, but have mostly focused on cognitive, electrophysiological, or imaging candidates in previous efforts (Glahn et al., 2014; Meyer-Lindenberg & Weinberger, 2006; Sanchez-Roige & Palmer, 2020). As with many neuropsychiatric disorders, the genetic architecture of GGE is complex, albeit heritability is comparably high (Section 1.2.2). This means that no particular combination of genes or environmental influences can characterize the diseased individual. It is conceivable that an endophenotype approach could lead to a greater discoverability of the genetic contribution than whole-genome or exome screenings in large populations. However, a few criteria need to be met for an endophenotype according to Gottesman that distinguish it from a classical biomarker (Gottesman & Gould, 2003). Prerequisites are a correlation of the marker with disease liability, heritability and co-inheritance in families. Furthermore, an endophenotype should be independent of the disease status. In concrete terms, this means

that an expression of the marker can be present in an individual without having the disease. However, this should be the case more often in non-affected family members than in the general population.

1.3.2 Endophenotypes in GGE

Early reports about endophenotypes in GGE concerned cognitive alterations. Iqbal et al. (2009) derived cognitive profiles in patients with GGE, healthy controls and healthy siblings of the patients. They found a tendency for the performance of patients and siblings in expressive language and higher-order executive functions to be similar but different from that of controls. Wandschneider et al. (2010) assessed prospective memory in 19 patients with JME, a GGE subtype, 21 siblings and 21 healthy individuals, reflecting the ability to execute previously planned intentions (Ellis, 1996). Again, subtle deficits were reported for the patients, and to a lesser extent also in the siblings. This evidence for genetic cognitive profiles in JME, albeit of small effect size, was further substantiated by Iqbal et al. (2015) for a number of other cognitive functions and similarly for a mixed GGE patient cohort (Chowdhury, Elwes, et al., 2014).

One of the first endophenotype studies using imaging in GGE focused on the most common GGE subtype JME (Wandschneider et al., 2014). During an fMRI working memory paradigm, coactivation of the primary and supplementary motor areas was altered in patients with JME and sustained with increasing demand, whereas deactivation occurred in healthy controls. Functional connectivity between motor and frontoparietal networks were also increased. Healthy siblings of the patients presented with a similar pattern. This study provided first important insights that atypical function and wiring of large-scale networks in JME may be a precursor mechanism of the disease. Furthermore, it suggested that this network behavior could underlie the observed cognitive deficits in JME (Wandschneider et al., 2014). This finding of a hyperactivated motor system in JME and siblings has been later extended to other cognitive domains such as memory and expressive language, suggesting a more domain-independent endophenotype (Caciagli et al., 2020). Based on fMRI findings, others argued for a state-independent hypersynchronous sensorimotor network as GGE endophenotype (Tangwiriyasakul et al., 2019), that is, for a generally increased level of spontaneous synchronization.

Chowdhury and colleagues provided further evidence for genetically influenced network conditions by studying 35 mixed GGE patients, 42 unaffected first-degree relatives, and 40 healthy individuals using conventional 10-20 scalp EEG (Chowdhury, Woldman, et al., 2014). Network measures characterizing the functional brain topology revealed stronger local clustering and mean variance of functional connections in the theta range (6-9 Hz) in the patients and relatives. Other evidence for altered wiring costs and network efficiency in GGE can be derived from a study on (structural) cortico-cortical connectivity in JME patients and siblings (Wandschneider et al., 2019). Cortical distance measures were increased in prefrontal areas, anterior cingulate, and temporo-polar cortices with effects on a wide range of well-known functional networks across the cortex (Wandschneider et al., 2019). Again, siblings had

similar topological patterns, corresponding to the note that these MRI markers can reflect neurodevelopmental processes and genetic organization of the cortex (Chen et al., 2013). Other morphological markers have also been looked into, such as curvature, surface area, and cortical thickness, for which regional differentiation is presumably controlled by gene expression along different topographical axes in the brain (Chen et al., 2013). Increased folding complexity of the cortical structures and surface area have been found to be present in JME patients and siblings in prefrontal and cingulate areas, whereas cortical thickness was only altered in the patients (Wandschneider et al., 2019). Also, morphological changes in the hippocampus and hippocampal malrotation were detected in the same study groups, with functional implications during verbal memory encoding, albeit without a behavioral (cognitive) correlate (Caciagli et al., 2019).

Overall, a handful of studies have investigated cognitive impairments in GGE and reported similar performance of the siblings, which gave rise to the question of a neurobiological substrate with heritable basis. In JME, (f)MRI paradigms have linked cognitive variables with brain function and structure with the possibility of using the employed markers as endophenotypes. At this point, it remains unanswered whether these findings are specific for JME or can be extended to other forms of GGE. Two other studies suggested that, independent of external or cognitive variables, there are basic heritable alterations in functional networks in GGE in the interictal state, specifically in sensorimotor regions and network organization. Given that rapid and abrupt changes in neuronal dynamics are characteristic to GGE and epilepsy in general, it is of utmost interest, to study oscillatory activity at high temporal resolution, as well as the underlying etiology leading to seizure susceptibility. Significantly, including first-degree relatives in the study also allows better delineation of disease causes from consequences or confounding factors such as pharmacological treatment.

1.3.3 Age at disease onset and brain oscillations during development

In the search for causative mechanisms for GGE using brain imaging, the age at disease onset may also provide important clues. Brain development and aging is critical in many pathological conditions and also in epilepsy. GGE subtypes are classically characterized by a specific window for age at onset (Vorderwülbecke et al., 2021). The onset of childhood absence epilepsy is usually between four and eight years of age with several absence seizures a day. With its often less frequent absences, juvenile absence epilepsy typically begins around 11 years and can include GTCS. In some cases, absences may also be present in JME with onset in adolescence. Myoclonic seizures are a hallmark of JME, but GTCS can also occur (Mullen et al., 2018). Generally speaking, in age-associated diseases, there could be a deviation from normative brain development or aging leading to the expression of symptoms and disease. Clinical symptoms may arise many years later than the presumably actual onset of disease as is for example suspected for Alzheimer's disease (Zvěřová, 2019) that primarily manifests during senescence.

Establishing biomarkers which quantify biological aging likely more meaningfully than the chronological age, has gained momentum in the last decade (Franke & Gaser, 2019). In

neuroimaging such markers have been mostly based on neuroanatomical data and are intended for individual health risk assessment and prediction (Franke & Gaser, 2019). In essence, the idea is to use imaging data to infer the deviation of an individual's brain age at a certain time point from that of a population norm. The relationship between chronological age and the expression of an imaging marker is studied using machine learning in a training dataset of healthy individuals, which then can be used for prediction in another sample (Cole & Franke, 2017). Usually, the difference between the individual computed brain age and the actual chronological age is calculated and referred to the 'brain age gap' or 'brain age delta' (Smith et al., 2020). This paradigm has been shown to have clinical relevance and has provided means to study the interaction of normal brain aging and pathology (Cole & Franke, 2017). Moreover, development and aging are complex processes, with central genetic and epigenetic determinants, and are marked with structural and functional brain changes (Higgins-Chen et al., 2021). It is conceivable that genetic determinants of lifespan trajectories are intertwined with genetic risk for disease. Remarkably, a study with over 45,000 individuals aged 3-96 years found signs of brain aging in common brain disorders using markers of structural MRI and machine learning. Moreover, the authors have demonstrated partial genetic overlap for "healthy" brain aging and disorders with clinical appearance in early or late life, including schizophrenia, bipolar spectrum disorder, autism spectrum disorder, attention-deficit hyperactivity disorder, multiple sclerosis, major depressive disorder, and Alzheimer's disease (Kaufmann et al., 2019). Although disease patterns cannot be expected to be fully congruent with signs of biological aging (Smith et al., 2020), a deeper understanding of dynamic lifespan trajectories may be an important building block in unravelling the complexity of age-related diseases (per se and specifically in epilepsy). In fact, recent investigations have challenged the claim that the brain age gap mainly reflects accelerated brain aging (Vidal-Pineiro et al., 2021). Instead, the authors have demonstrated that these markers rather reflect early-life factors than longitudinal changes in an individual's brain. In their study, they compared longitudinal and cross-sectional estimates of the brain age in two independent, large-scale data sets and were unable to determine a relationship between them. Remarkably, cross-sectional brain age gap was associated with self-reported birth weight and genetic composition related to an 'older looking brain'. These findings strongly imply that genetic factors have, probably in interaction with environmental influences, a stable and long-lasting effect on these brain markers, in this case structural MRI markers (Vidal-Pineiro et al., 2021). So far, most brain age studies have been based on a single global marker of brain morphology. Recently, others have used more sophisticated procedures and calculated about 3,900 imaging phenotypes and aggregated them into so-called "modes" based on their co-variation across age as well as overarching clusters (Smith et al., 2020). The idea is that the combined phenotypes represent specific biological processes. For example, altered white-matter structure could index axonal degeneration. Interestingly, brain age gaps for these modes could be linked to the genetic architecture of individuals, which was not possible with a single (unimodal) brain age measure for an individual (Smith et al., 2020). In this sense, it seems valuable to consider meaningful and distinct entities composed of multiple and multimodal features to understand what causes deviations from the norm.

Roughly speaking, the brain age approach could, in future, be a window into prediction of brain conditions at a certain time point in a (diseased) individual and allow inferences about genetic determinants and/or early developmental parameters. Given that the pathological dynamics in epilepsy are well represented at faster timescales using electrophysiology techniques, information about fast oscillatory activity across the lifespan could greatly help to disentangle disease-related effects from normal effects of aging. However, as opposed to research investigating brain structural features or slow-waves using fMRI, there is little scientific evidence for how features of fast brain oscillations evolve with age, nor is much known about the biological basis of such markers. Therefore, as a first step, this should be sought in healthy controls before drawing conclusions about individuals with epilepsy.

1.4 Methods of Brain Electrophysiology

In the original studies of this dissertation, I will focus on the use of EEG and MEG to study brain activity and synchronization of GGE, as well as the characterization of markers used across the lifespan. This section will provide a brief introduction to the origin of brain oscillations in MEG and EEG, connectivity measures, and source imaging in epilepsy.

1.4.1 Oscillatory activity and synchronization

The electrical or magnetic signals measured at the scalp using EEG and MEG, respectively, are generated by neurons that are active in a temporally coordinated manner. In order for the signals to be observable from outside the brain, an assembly of several thousands of neurons must be functionally and spatially organized (Da Silva, 2013). This is the case with pyramidal neurons in the cortex, which are organized in columns with the dendrites parallel to each other. Excitation of postsynaptic neurons lead to intracellular and extracellular current flows, producing an electric field along the main axis of the neurons, and perpendicular to it, a magnetic field that can be measured from a distance (Berger, 1929; Cohen, 1972; Da Silva, 2013; Hämäläinen et al., 1993). The main feature of electrophysiological recordings is the presence of oscillations of different rhythms reflecting variations in the excitability of (local) neuronal populations (Buzsáki & Wang, 2012; Cohen, 2017). The signal strength, that is, amplitude, and timing of the oscillations also vary, which likely facilitates information flow between network nodes if synchronized (Womelsdorf et al., 2007) and enables long-range communication in the brain (Wang, 2010). The functional relevance of neuronal oscillations has been the subject of *in vitro* and *in vivo* experiments (Cohen, 2017) as well as cross-species studies for decades (Buzsáki et al., 2013). The basic idea is that the study of neuronal oscillations can provide insights into mechanistic principles of the brain, both in task-based study paradigms, and at rest in healthy subjects as well as in pathological conditions such as in epilepsy (Berger, 1929; Buzsaki, 2006; Cohen, 2017; Singer, 1999; Uhlhaas & Singer, 2006; Varela et al., 2001).

Epileptic seizures are reflected in electrophysiological recordings as high-amplitude, often rhythmic activity, and are interpreted as aberrant neuronal excitability and synchronization (Jiruska et al., 2013). Initially, this state was termed “hypersynchronous” (Penfield & Jasper, 1954), however, it has become clear that synchronization in epilepsy is much more complex (Jiruska et al., 2013). Using EEG or MEG, a number of different metrics are available to study synchronization in the brain (Varela et al., 2001; Wang et al., 2014), usually grouped under the umbrella term “connectivity”. Functional connectivity represents the statistical interdependence between time series (Friston et al., 1993), with the possibility of also considering the direction of interaction (Bastos & Schoffelen, 2016). In the time domain these include correlation metrics or, in the directed case, cross-correlations, Granger causality, or transfer entropy (Bastos & Schoffelen, 2016). Of particular interest is often the study of neuronal interactions in specific temporal rhythms, such as in the study of epilepsy, which requires the transformation of signals into the frequency domain. Most metrics quantify the

consistency of phase differences between oscillating signals. Others ignore phase relations between the signals and use only the amplitude or envelopes to study mutual dependencies (Hipp et al., 2012). It is worth noting that there is no universally preferred connectivity metric, as all have their advantages and disadvantages. The choice is usually difficult and depends on factors such as applicability, comparability or accessibility within a research community (Bastos & Schoffelen, 2016). Throughout this work, we used the imaginary part of coherency (Nolte et al., 2004), which shall briefly be introduced. Coherence quantifies the phase synchrony in the frequency domain and is equivalent to the cross-correlation function in the time domain. In the case of two signals this is achieved by multiplying the amplitudes of the signals and subtracting their phases, resulting in the cross-spectrum between the signals (Bastos & Schoffelen, 2016). Now, studying connectivity at the human scalp sensors introduces one serious problem. The activity of a single source in the brain is picked up by many sensors, and therefore the relationship between sensor signals does not reflect true connectivity of brain sources. To mitigate this problem, the complex-valued coherency is projected onto the imaginary axis so that connectivity with a phase-lag of zero or two π, which is considered spurious, is discarded. The spread of activity from one source to multiple sensors or volume conduction has no time delay and therefore a zero-phase lag. Neural transmission, on the other hand, should result in amplitude correlation and additionally in a time delay, which is reflected in a phase shift. In essence, the imaginary part of coherency is sensitive only to time-lagged synchronization of signals (Nolte et al., 2004).

1.4.2 Source imaging and the use of EEG and MEG in epilepsy

Besides measuring signal properties and the topographical distribution given a set of sensors outside the brain, it is usually of interest to know about the signal's origin. However, this is not straightforward because electromagnetic fields of a single source spread spatially (Helmholtz, 1853) and may be picked up by multiple sensors. Conversely, one sensor on the scalp may record the activity of multiple neuronal sources (Fender, 1987). Essentially, the question is which functional and anatomical configurations in the brain result in the sensor topographies measured using EEG or MEG, which, without constraints, has an unlimited number of solutions (Da Silva, 2013). To solve this so called "inverse problem", several assumptions need to be made and parameters set, which may have an impact on the results (Fender, 1987; Michel et al., 2004). In general, using mathematical descriptions and projection methods one can determine the anatomical localization of cortical sources with centimeter precision (Cuffin et al., 2001; Fuchs et al., 2002; Klamer et al., 2015). One concept in source-space imaging is the forward model, which includes the anatomy of an individual brain or a template brain (head model), the locations of the EEG/MEG sensors, and a set of sources or dipoles (Michel et al., 2004). Conceptually, the forward solution tells us what the activity at the sensors looks like given a set of activated sources in the brain and the anatomical conditions (Cohen, 2017; Michel et al., 2004). In the case of EEG, signals traveling quasi-instantaneously from the brain to the scalp are distorted by several layers of cortical tissue of different electrical conductivities leading to an attenuation of the electrical signal. This must,

therefore, be accounted for in the forward solution by modeling the cortical layers, that is, by assuming specific conductance values for brain tissue (Michel et al., 2004). Head models can be simplistic or complex by including skin, skull, cerebrospinal fluid, gray and white matter (Wolters et al., 2006). Conversely, these tissues are constantly permeable to magnetic signals, which means they are less affected by anatomical features and require less complex modeling (Da Silva, 2013; Hansen et al., 2010). Assumptions are also made about the density and localization of dipoles, which can be generated based on an MRI volume or cortical surface area (Saad & Reynolds, 2012). Once the forward model is computed, the unknown sources corresponding to the MEG/EEG data are estimated by defining a set of sensor weights for each given source point in the brain. Several source imaging methods exist with fixed weights or adaptive filters such as beamforming methods (Cohen, 2017; Michel et al., 2004). In this way, an approximate solution to the inverse problem is found that provides information about where in the brain the measured EEG or MEG might have originated.

In epilepsy, source imaging has been of special interest for the localization of epileptic discharges and seizure foci for the purpose of more accurate resection in focal epilepsies. In GGE, most of the studies have reported on EEG power at the sensor level without mapping it to brain sources and often, EEG systems with comparably low sensor density (< 64 sensors) were used (Faiman et al., 2021). Today, many high-density systems with 128 or 256 sensors are available, which can yield improved resolution if noise is kept low (Da Silva, 2013). EEG reflects relative changes in electrical potentials between an electrode and, typically, a reference electrode in microvolts (Cohen, 2017). MEG usually comes with a whole-brain coverage of at least 64 sensors and is a reference-free method (Hansen et al., 2010), measuring brain activity at the units of femtotesla (10^{-15} tesla). EEG and MEG are thought to reflect the same neuronal phenomena (Da Silva, 2013), but due to their unique signal properties and associated modeling aspects, the two methods are complementary (Malmivuo, 2012), which will be covered in more detail in Chapter 4.2 of this dissertation. Although MEG has been shown to yield additional information in epileptic spike detection (Heers et al., 2010; Rampp et al., 2019), resting-state MEG studies in GGE are scarce (Elshahabi et al., 2015; Krzemiński et al., 2020; Li Hegner et al., 2018; Lopes et al., 2021).

2 Scope of the Dissertation

Epilepsy, including genetic generalized epilepsy, is increasingly understood as a network disease, and imaging and electrophysiological methods have contributed to its more sophisticated understanding. Recordings of epileptic seizures and epileptic discharges show rapid, rhythmic synchronization of brain activity and are essential features for the classification of seizure types and syndromes. However, how large-scale brain networks behave in GGE during periods without epileptiform activity, that is, during the interictal state, is not well understood and must be clarified. Are functional networks altered in epilepsy patients in general, as a sign of the disease, and not just during a seizure? It is further essential to grasp what is being measured when examining the interictal state by means of resting-state measurements — do the corresponding markers reflect disease mechanisms, disease progression, or even treatment effects? And, most intriguingly, how is the genetic etiology of GGE related to brain activity and synchronization during this state? Conversely, do electrophysiological markers of activity and synchronization map disease mechanisms that may be genetically influenced? In addition to the conceptual description of the interictal state, it is pertinent whether MEG and HD-EEG provide complementary information about network alterations in GGE and whether these correspond to known subtle structural changes. This is critical for a better understanding of the disease and the imaging phenotype in GGE, which could support diagnostics and treatment in future. Moreover, it is of significance whether electrophysiological markers change across the lifespan and what this might reveal about developmental aspects relevant to GGE.

Overall, I address the following overarching questions in this dissertation:

(A) Are markers of brain oscillatory activity and synchronization during the interictal state able to capture disease variations related to the genetic makeup of GGE? Can a multimodal assessment of functional and structural properties of the brain enhance the understanding of the network phenotype in GGE?

(B) How are electrophysiological markers expressed in different age groups of healthy individuals? Do they reflect brain development and aging processes, and how might this relate to disease trajectories in GGE?

To answer the questions in (A), we first investigated power and connectivity in patients with GGE and their healthy siblings at rest using MEG (Study I, Chapter 4.1). In a second step, we applied the same analysis principles to the HD-EEG data obtained from the same study cohort. Further, we statistically combined the MEG and HD-EEG results with cortical thickness measures in a joint analysis (Study II, Chapter 4.2). For the questions in (B), we used MEG data from a large-scale research project to study how power and connectivity evolve from early adulthood into old age (Study III, Chapter 4.3).

3 Materials and Data

Multimodal imaging in genetic generalized epilepsy

For the studies presented in Chapter 4.1, Chapter 4.2, and Other Contributions, the data were measured and analyzed as part of a multimodal imaging project. A patient sample with mixed GGE subtypes was recruited through the Department of Neurology, University Hospital of Tübingen, Germany. Further, healthy siblings of the patients and healthy controls were contacted and interviewed regarding medical history and eligibility for MR examinations. Individuals with neurologic or psychiatric disorders, cardiac or respiratory diseases, and medication intake were excluded. All eligible individuals were invited to participate in the study at the MEG Center, University of Tübingen, and at the Department of Biomedical Magnetic Resonance, University of Tübingen, Germany. All individuals were measured in a magnetically shielded room during eyes-closed resting-state in supine position using MEG (30 min) and separately using HD-EEG (30 min). After a short break, the patients with GGE and the siblings were neuropsychologically examined (approximately 60 minutes). The protocol was based on an established presurgical paradigm of the department and included tests covering different executive memory and visuo-spatial functions, attention, and fluid and crystallized intelligence. Finally, we conducted simultaneous fMRI-EEG measurements (30 min) and anatomical and diffusion tensor imaging (T1 weighted/T2-FLAIR/DTI; 30 min).

My contributions: I evaluated and documented the medical reports of the patients. I coordinated the study, contacted the patients and their healthy siblings, and healthy controls, interviewed them concerning medical history and MR eligibility. I acquired the MEG, HD-EEG, fMRI-EEG and MRI data of 20 patients, 21 siblings and 20 healthy individuals, and conducted and evaluated the neuropsychological assessments. I was also involved in data curation and documentation.

Lifespan trajectory of oscillatory markers

The study of electrophysiological markers across the lifespan (Chapter 4.3) is based on the data repository of a large-scale collaborative research initiative of the Cambridge Centre for Ageing and Neuroscience (Cam-CAN). Launched in 2010, the project aims at studying how cognitive abilities can be retained into old age. Cam-CAN provides cross-sectional epidemiological, cognitive, and neuroimaging data derived from two study stages. In a first stage, 3,000 adults ranging from 18 to 88 years of age were surveyed as to health and lifestyle and were cognitively tested. In a second stage, 700 of them underwent task and resting-state fMRI and MEG measurements. Again, the individuals were assessed using a cognitive test battery. Individuals with cognitive impairments, communication difficulties (hearing and vision), mobility problems, medical conditions, and substance abuse were excluded (Shafto et al., 2014; Taylor et al., 2017).

My contributions: I applied for data access, downloaded and processed the data.

4 ORIGINAL STUDIES

My research contributions for this dissertation have been summarized into the following three main manuscripts:

4.1 Heritability of magnetoencephalography phenotypes among patients with genetic generalized epilepsy and their siblings

Authors: Christina Stier, Adham Elshahabi, Yiwen Li Hegner, Raviteja Kotikalapudi, Justus Marquetand, Christoph Braun, Holger Lerche, and Niels K. Focke

CS and NKF designed and conceptualized the study; CS, AE, RK, JM acquired data; CS analyzed the data, CS performed statistical analyses and visualization; CS and NKF interpreted the results; YLH and CB provided critical input on the methods; NKF and CB supervised the project; CS wrote the manuscript; NKF, AE, YLH, RK, JM, CB, HL revised the manuscript for intellectual content

Published in *Neurology*, 2021

4.2 Combined electrophysiological and morphological phenotypes in patients with genetic generalized epilepsy and their healthy siblings

Authors: Christina Stier, Markus Loose, Raviteja Kotikalapudi, Adham Elshahabi, Yiwen Li Hegner, Justus Marquetand, Christoph Braun, Holger Lerche, and Niels K. Focke

CS and NKF designed and conceptualized the study; CS, RK, AE, JM acquired data; CS and ML analyzed the data, CS performed statistical analyses and visualization; CS and NKF interpreted the results; YLH and CB provided critical input on the methods; NKF and CB supervised the project; CS wrote the manuscript; NKF, ML, RK, AE, YLH, JM, CB, HL revised the manuscript for intellectual content

In revision for resubmission to *Epilepsia*; version dated January 28, 2022

4.3 Lifespan trajectory of oscillatory power and phase

Authors: Christina Stier, Christoph Braun, Niels K. Focke

CS and NKF designed and conceptualized the study; Cam-CAN acquired and provided data; CS analyzed the data, CS performed statistical analyses and visualization; CS and NKF interpreted the results; CB provided critical input on the methods; NKF supervised the project; CS wrote the manuscript; NKF revised the manuscript for intellectual content

In preparation for submission.

4.1 HERITABILITY OF MAGNETOENCEPHALOGRAPHY PHENOTYPES AMONG PATIENTS WITH GENETIC GENERALIZED EPILEPSY AND THEIR SIBLINGS

RESEARCH ARTICLE OPEN ACCESS

Heritability of Magnetoencephalography Phenotypes Among Patients With Genetic Generalized Epilepsy and Their Siblings

Christina Stier, MSc, Adham Elshahabi, MD, MSc, Yiwen Li Hegner, MD, PhD, Raviteja Kotikalapudi, PhD, Justus Marquetand, MD, Christoph Braun, PhD, Holger Lerche, MD, and Niels K. Focke, MD

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Correspondence
Dr. Focke
niels.focke@
med.uni-goettingen.de

Abstract

Objective

To assess whether neuronal signals in patients with genetic generalized epilepsy (GGE) are heritable, we examined magnetoencephalography resting-state recordings in patients and their healthy siblings.

Methods

In a prospective, cross-sectional design, we investigated source-reconstructed power and functional connectivity in patients, siblings, and controls. We analyzed 5 minutes of cleaned and awake data without epileptiform discharges in 6 frequency bands (1–40 Hz). We further calculated intraclass correlations to estimate heritability for the imaging patterns within families.

Results

Compared with controls (n = 45), patients with GGE (n = 25) showed widespread increased functional connectivity (θ to γ frequency bands) and power (δ to γ frequency bands) across the spectrum. Siblings (n = 18) fell between the levels of patients and controls. Heritability of the imaging metrics was observed in regions where patients strongly differed from controls, mainly in β frequencies, but also for δ and θ power. Network connectivity in GGE was heritable in frontal, central, and inferior parietal brain areas and power in central, temporo-parietal, and subcortical structures. Presence of generalized spike-wave activity during recordings and medication were associated with the network patterns, whereas other clinical factors such as age at onset, disease duration, or seizure control were not.

Conclusion

Metrics of brain oscillations are well suited to characterize GGE and likely relate to genetic factors rather than the active disease or treatment. High power and connectivity levels co-segregated in patients with GGE and healthy siblings, predominantly in the β band, representing an endophenotype of GGE.

From the Clinic of Clinical Neurophysiology (C.S., R.K., N.K.F.), University Medical Center Göttingen; Department of Neurology and Epileptology, Hertie Institute of Clinical Brain Research (C.S., A.E., Y.L.H., R.K., J.M., H.L., N.K.F., C.B.), and MEG Center (C.B.), University of Tübingen, Germany; Department of Neurology (A.E.), University Hospital Zurich; Institute of Psychology (R.K.), University of Bern, Switzerland; and CIMeC (C.B.), Center for Mind/Brain Sciences, University of Trento, Italy.

Go to Neurology.org/N for full disclosures. Funding information and disclosures deemed relevant by the authors, if any, are provided at the end of the article.

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Glossary

FWE = familywise error correction; **GGE** = genetic generalized epilepsy; **GSWD** = generalized spike-wave discharges; **ICC** = intraclass correlation; **JME** = juvenile myoclonic epilepsy; **MEG** = magnetoencephalography; **SNR** = signal-to-noise ratio.

Idiopathic/genetic generalized epilepsy (GGE) is a common epilepsy syndrome accounting for 15%–20% of all epilepsies.[1] Different seizure types can occur, including absence, myoclonic, and generalized tonic-clonic seizures.[2] For GGE, a polygenic background is presumed.[3] So far, gene discovery has been scarce despite high heritability[4] and large-scale collaborative efforts.[3,5] Thus, it is of high interest to seek subclinical traits of the syndrome (endophenotypes) that reflect the genetic background of the disease and cosegregate in families with affected individuals.[6] Candidate markers for GGE have been proposed, such as cognitive functioning,[7-9] frontal lobe[10] and hippocampal morphology, hippocampal function,[11] and functional network topology.[12] Furthermore, patients with juvenile myoclonic epilepsy (JME) and their siblings have shown increased activation of the motor system during cognitive tasks.[13,14] However, increased brain connectivity and power has also been found in absence of cognitive load in a mixed GGE cohort and in widespread regions.[15,16] It is less clear to which extent observed findings reflect disease activity, effects of seizure burden, or treatment. Also, various methodologic approaches hinder reproducibility and comparability of functional network studies.[17] Given that characteristics of spontaneous brain oscillations at rest are heritable,[18,19] the studies of unaffected siblings may help to disentangle genetic factors from secondary disease effects.

This study set out to assess whether imaging metrics based on oscillatory neural activity and measured by magnetoencephalography (MEG) during resting-state could represent an endophenotype of GGE.

Methods

Standard Protocol Approvals, Registrations, and Patient Consents

This study was approved by the local ethics committee of the Medical Faculty of the University of Tübingen and conducted in compliance with the principles of the Declaration of Helsinki. All participants provided written informed consent.

Recruitment

Patients with GGE and their siblings were consecutively recruited through the clinical database of the Department of Neurology, University Hospital of Tübingen, Germany, between 2013 and 2019. Advertisement and recruitment of controls was conducted in the local area. All patients were diagnosed with GGE according to the recent International League Against Epilepsy classification.[2] At the time of the study, siblings and controls never had experienced seizures, were free of any neurologic and psychiatric diseases, and did not take any medication.

MEG Recording

Resting-state data were measured in supine position (275 channels system, CTF Inc.) in the MEG center of the University of Tübingen (585.9 Hz sampling rate). Participants underwent 30 minutes of continuous recording in order to have sufficient data after exclusion of segments with generalized spike-wave discharges (GSWD). All participants were instructed to relax, to keep their eyes closed, not to fall asleep, and not to think of anything in particular.

Individual Head Anatomy

A sagittal high-resolution T1-weighted image was acquired for all participants (3D-MPRAGE, repetition time = 2.3 seconds, echo time = 3.03 ms, flip angle = 8°, voxel size = 1 × 1 × 1 mm), either on a Siemens Magnetom Trio 3T scanner equipped with a 12-channel head coil (11/45 controls, 5/25 patients) or at the Siemens Magnetom Prisma 3T system (Siemens AG) with a 64-channel head coil (34/45 controls, 18/18 siblings, 20/25 patients). Detailed description of processing methods and references can be found elsewhere.[20] In brief, individual cortical surfaces were reconstructed using FreeSurfer (surfer.nmr.mgh.harvard.edu/) and further subjected to SUMA (afni.nimh.nih.gov/download/). SUMA decimated each participant's cortical surface to 1,002 common vertices per hemisphere. The surface was resampled using the fsaverage template (FreeSurfer) and SUMA (ld = 10). In addition, 6 subcortical nuclei (bilateral amygdala, hippocampus, thalamus, caudate, putamen, and pallidum) were reconstructed based on the fsaverage template. Each region was converted to surfaces and spatially normalized to Montreal Neurological Institute space (DARTEL; SPM12; fil.ion.ucl.ac.uk/spm/software/spm12/) using CAT12 DARTEL template (neuro.uni-jena.de/cat/). This procedure eventually yielded 2,338 vertices for each participant and point-for-point anatomical correspondence for cortical and subcortical regions. Finally, the individual cortical mesh was realigned to the CTF sensor space using the fiducial positions recorded during the MEG session. A volume conduction head model was constructed for the MEG source analysis using the single shell method implemented in Fieldtrip.

MEG Data Processing and Source Analysis

Preprocessing and further analysis steps were performed using Fieldtrip (fieldtriptoolbox.org/) running in MatLab (version 9.0, R2016a, Mathworks Inc.) as described and referenced elsewhere.[20] In short, data were preprocessed (Butterworth band-pass filter 1–70 Hz, line-noise removal), downsampled (150 Hz), and cut into epochs of 10 seconds

length. Trials with GSWD were manually marked and excluded from the further analysis including one trial preceding and one trial after the event (±10 seconds). Each trial was visually inspected and trials with artefacts were manually removed (e.g., movements, excessive muscle activity, sensor jumps). We used independent component analysis to detect and manually reject cardiac and eye movement artefacts. All trials were again reviewed and vigilance was rated according to sleep scoring criteria of the American Academy of Sleep Medicine. Thirty trials of cleaned and awake data (300 seconds) per participant were randomly selected for source analysis. We performed spectral analysis on the MEG sensor data using a multitaper fast Fourier time-frequency transformation approach with frequency-dependent discrete prolate spheroidal sequences tapers for 6 frequency bands (δ: 2 ± 2 Hz, θ: 6 ± 2 Hz, α: 10 ± 2 Hz, β1: 16 ± 4 Hz, β2: 25 ± 4 Hz, and γ: 40 ± 8 Hz). Power and the cross-spectral density were derived from the Fourier transformed sensor-level data. We used beamforming (dynamic imaging of coherent sources) to project the data to the source space. For each vertex point of the individual cortical mesh, the lead field matrix was calculated and an adaptive spatial filter was applied separately for each frequency band (regularization: λ = 5%). Power was computed for each source position. The coherency coefficient, which quantifies phase synchrony between 2 signals, was estimated between all pairs of sources (n = 2,338). We then investigated the absolute imaginary part of coherency to reduce contributions to the connectivity estimate, which are due to potential field spread.[21] In sum, a symmetrical, weighted, and undirected individual functional connectivity matrix was constructed for each frequency band. We averaged the weights of each vertex to estimate the overall connection strength of a vertex. To obtain an overall indicator of metrics, we also averaged connectivity and power across all vertices, yielding one global value per participant.

Statistical Analysis of Imaging Metrics

Group differences in power and connectivity were assessed using Permutation Analysis of Linear Models (fsl.fmrib.ox.ac.uk/fsl/fslwiki/PALM), a nonparametric statistical tool. In order to allow permutation inference in presence of possible dependence structures among related participants, we applied multilevel block permutation.[22] Blocks of exchangeable units were defined and shuffled as a whole (families and single unrelated participants). Observations within a block were rearranged among themselves (observations within families). We carried out single t contrasts instead of an overall F test, which would have only allowed limited exchangeability of the data given related family structures (patients/siblings) and unrelated controls. Based on previous work,[15,16] we hypothesized increased network levels in patients with GGE compared with controls and thus ran one-sided comparisons (controls < siblings, siblings < patients, controls < patients). Groups were contrasted vertex-based and on a global level, respectively, and for each frequency band separately. For each comparison, a general linear model was fit for every permutation, with imaging metrics as dependent variables. Group association and age constituted the predictors. Sex was initially included as additional predictor in the model but did not change any of the main results and was not further considered in the analyses. The data were permuted 5,000 times. An estimate of the empirical distribution of the t statistics under the null hypothesis was constructed, from which the p values were generated. In the vertex-based analysis, we corrected for multiple comparisons on cluster level using threshold-free cluster enhancement.[23] p Values were familywise error corrected (FWE) within each group contrast and indicated as –log10 p with a significance threshold of 1.3 ($p < 0.05$). Effect sizes (Cohen d) for vertex-based and global group comparisons were derived from the t values of the linear models. d Is therefore adjusted for age effects. An effect size of $d = 0.2$ is considered to be small, $d = 0.5$ intermediate, and $d = 0.8$ large.[24]

Heritability of Imaging Patterns

We explored the extent to which imaging phenotypes are heritable and quantified this using intraclass correlation (ICC) through linear mixed-effects modeling.[25] ICC values were estimated based on the random effect components of a mixed model, which allows the incorporation of confounding effects. Here, a mixed model was constructed for power and connectivity, respectively, as dependent variables, family membership as random effect alongside group (patients vs siblings), and age as subject-level covariate (fixed-effect). ICC(1,1) was computed based on the variances of the random effect (family) and the total random effect variance (family and residual variance)[25] using R (nlme package; CRAN.R-project.org/package=nlme) and restricted maximum-likelihood estimation. ICC ranges from 0 to 1, where an ICC close to 1 indicates correlated connectivity and power levels for patient–sibling pairs in a family. A low ICC means that family affiliation is not relevant and thus genetic contribution is unlikely. In total, 14 GGE families contributed to the ICC estimations. We performed a regional resampling of the vertex-level metrics using the Desikan-Killiany atlas[26] to improve the signal-to-noise ratio (SNR) and calculated ICCs for 80 anatomically defined cortical and subcortical regions. Finally, we investigated whether heritability estimates of imaging metrics are particularly high in brain areas where patients show stronger differences from controls. To this end, effect sizes (Cohen d) from the vertex-wise group comparisons were averaged for each anatomical region and related to the ICC maps using Spearman rank correlation for each frequency band and metric. Higher positive correlations of effect sizes and ICC values imply genetic contribution to disease-related patterns in GGE.

Data Availability

All relevant data including power and connectivity results and ICC estimations are available from the corresponding author upon request. Raw imaging data are not publicly available due to data protection regulations.

Results

Participants

Twenty-eight patients with GGE, 21 siblings, and 50 controls underwent resting-state measurements. We excluded participants due to technical problems during the acquisition (n = 5), movement artifacts (n = 4), or sleep (n = 2), leaving datasets of 25 patients, 18 siblings (related to 15 patients), and 45 controls for further analysis. Raw data from 6 patients were also used in a previous study.[15] Anatomical MRI scans were visually rated as normal in all controls, siblings, and in 22 patients. Three patients had nonspecific findings (2 uncomplicated cysts, a single unspecific white matter lesion). Demographics and clinical details are described in the table. Family membership and GGE syndromes are indicated in figures 1A and 2A. The groups were comparable for age (analysis of variance, $p = 0.95$) and sex (χ^2, $p = 0.84$).

Connectivity Analysis

Compared with controls, patients with GGE showed increased functional connectivity in most of the frequency bands studied. Global connectivity (figure 3A) was higher in the θ ($t_{67} = 2.17$, $p = 0.011$, $d = 0.54$), α ($t_{67} = 2.36$, $p = 0.016$, $d = 0.59$), β1 ($t_{67} = 3.35$, $p = 0.0004$, $d = 0.84$), β2 ($t_{67} = 2.40$, $p = 0.023$, $d = 0.60$), and γ band ($t_{67} = 3.17$, $p = 0.008$, $d = 0.79$), but not in δ ($t_{67} = 0.88$, $p = 0.152$, $d = 0.22$). Vertex-based comparisons showed widespread bilateral increases across the frequency spectrum (figure 3B). Strongest effects were observed in the β1 frequency band with a focus on left-hemispheric temporal, frontal, central, and parietal regions. Mesio-frontal regions were also pronounced in α, β2, and γ frequency bands and postcentral regions mainly in the θ band. Connectivity of siblings statistically fell between patients and healthy controls (figure 3A). Global connectivity of patients was higher than in siblings for α ($t_{40} = 1.67$, $p = 0.047$, $d = 0.52$), β2 ($t_{40} = 1.98$, $p = 0.027$, $d = 0.61$), and γ ($t_{40} = 1.89$, $p = 0.044$, $d = 0.59$), but there were no significant differences in the remaining frequency bands (δ: $t_{40} = 1.38$, $p = 0.098$, $d = 0.43$; θ: $t_{40} = 1.19$, $p = 0.247$, $d = 0.37$; β1: $t_{40} = 0.90$, $p = 0.205$, $d = 0.28$). Siblings did not significantly differ from controls (δ: $t_{60} = -0.8$, $p = 0.444$, $d = -0.21$; θ: $t_{60} = 0.49$, $p = 0.223$, $d = 0.14$; α: $t_{60} = 0.45$, $p = 0.267$, $d = 0.13$; β1: $t_{60} = 1.59$, $p = 0.101$, $d = 0.44$; β2: $t_{60} = 0.46$, $p = 0.250$, $d = 0.13$; γ: $t_{60} = 0.749$, $p = 0.520$, $d = 0.21$). On a vertex level (figure 3C) and after correction for multiple comparisons, siblings differed from patients with GGE in α and β2 frequency bands ($p_{FWE} < 0.05$), but not from controls ($p_{FWE} > 0.05$).

Power Analysis

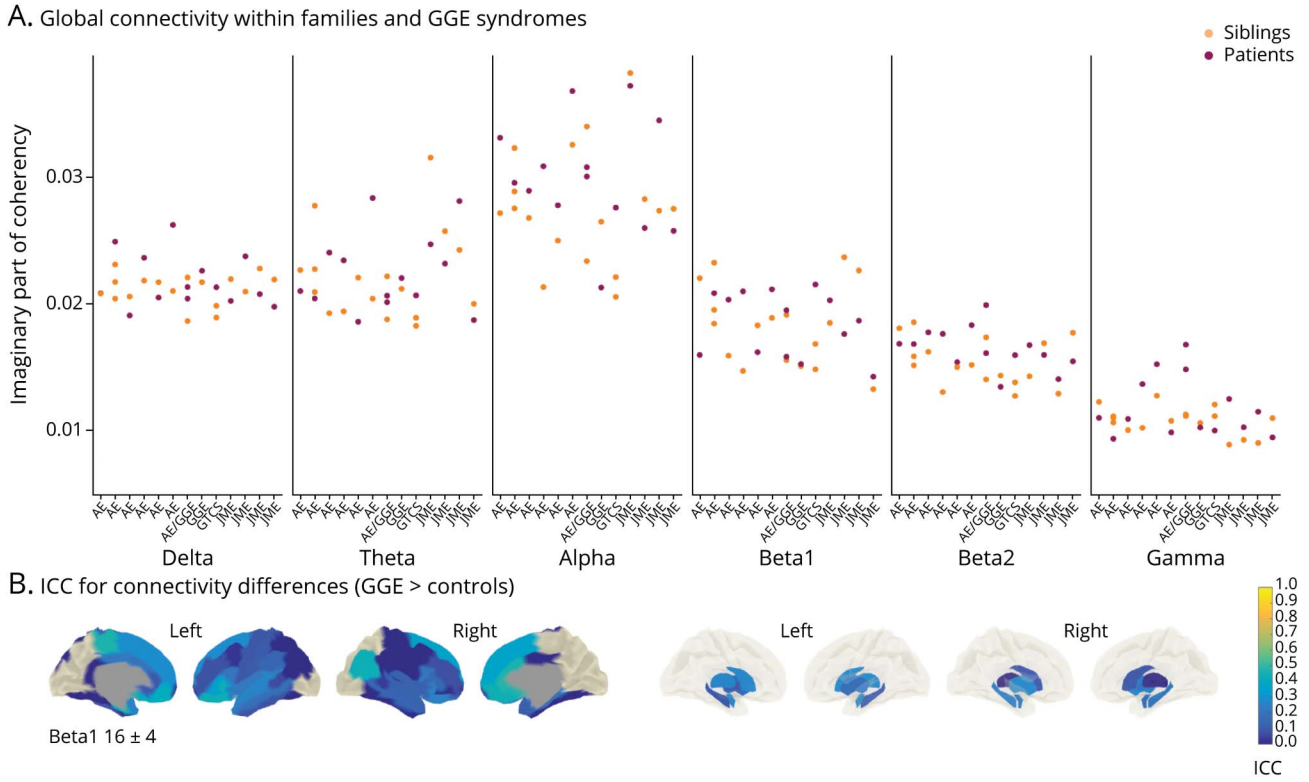
Patients with GGE had higher power than controls in all frequency bands studied, in the global (figure 4A; δ: $t_{67} = 3.15$, $p = 0.002$, $d = 0.79$; θ: $t_{67} = 3.73$, $p = 0.0002$, $d = 0.93$;

Table Study Population

	Patients	Siblings	Controls
Total	25	18	45
Female	16 (64)	10 (55)	28 (62)
Age, y	25 (22–37)	26 (22–42)	25 (23–35)
Positive family history of epilepsy/seizures	12 (48)	7 (39)[a]	0 (0)
GSWD during MEG recordings	9 (36)	0 (0)	0 (0)
GSWD in routine/long-term EEG	22 (88)	—	—
Seizure free >12 months	16 (64)	—	—
Drugs at measurement	1.2 (0–3)	—	—
Epilepsy syndrome		—	—
CAE	5 (20)	—	—
JAE	6 (24)	—	—
JME	5 (20)	—	—
GTCS	4 (16)	—	—
GGE	5 (20)	—	—
Age at onset, y	15 (10–17)	—	—
Disease duration, y	17 (8–24)	—	—

Abbreviations: CAE = childhood absence epilepsy; GGE = genetic generalized epilepsy (unclassified); GSWD = generalized spike-wave discharges; GTCS = generalized tonic-clonic seizures; JAE = juvenile absence epilepsy; JME = juvenile myoclonic epilepsy; MEG = magnetoencephalography.
Values are n (%), median (interquartile range), or mean (range).
[a] Positive family history beyond the related index patient with GGE.

Figure 1 Connectivity Within Families Related to Genetic Generalized Epilepsy (GGE) Imaging Patterns



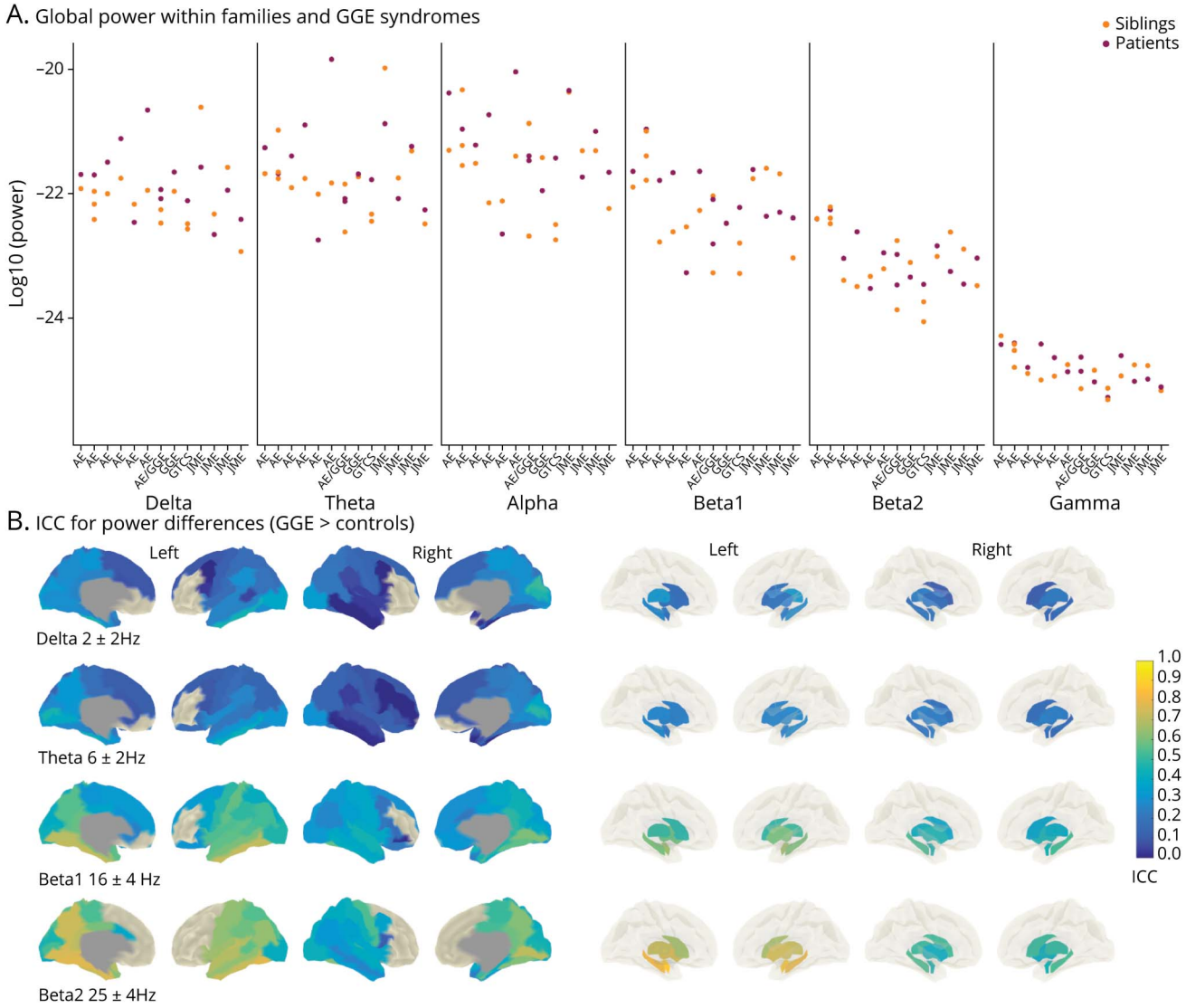
(A) Individual global connectivity values of patients with GGE and siblings are plotted with regard to their family membership (columns of data within frequency bands). Data of patients with GGE without a corresponding sibling are not shown. GGE syndromes of patients in each family are indicated on the x-axis. Childhood absence epilepsy and juvenile absence epilepsy are referred to absence epilepsies (AE). (B) Color-coded heritability estimates (intraclass correlation [ICC] values) per region based on the Desikan-Kiliany atlas[26] with small to large group-level differences between patients with GGE and controls (Cohen $d > 0.2$). Only ICC maps with a positive and significant correlation with averaged effect sizes are shown ($p < 0.05$). The color coding indicates the strength of ICC values in those regions for connectivity differences. ICC estimates were derived from random effect components of mixed models for each region, taking group and age effects into account. A large ICC indicates correlated imaging patterns for patient–sibling pairs in a family (n = 14) and thus heritability of the metrics. Cortical regions are displayed in the left column and subcortical regions are shown separately in the right column of the plot. GTCS = generalized tonic-clonic seizures; JME = juvenile myoclonic epilepsy.

α: $t_{67} = 4.22$, $p = 0.0002$, $d = 1.05$; β1: $t_{67} = 5.44$, $p = 0.0002$, $d = 1.36$; β2: $t_{67} = 4.24$, $p = 0.0002$, $d = 1.06$; γ: $t_{67} = 3.60$, $p = 0.0006$, $d = 0.90$) and vertex-based analysis ($p_{FWE} < 0.05$; figure 4B). Differences were focused on occipital-parietal and temporal regions. Also, hippocampal (α, β1, β2) and subcortical structures such as thalamus and putamen (β1) showed higher power. Patients also exhibited higher global power than siblings (figure 4A) in α ($t_{40} = 2.40$, $p = 0.027$, $d = 0.74$), β1 ($t_{40} = 1.34$, $p = 0.024$, $d = 0.41$), and γ ($t_{40} = 1.74$, $p = 0.048$, $d = 0.54$), but not in δ ($t_{40} = 1.96$, $p = 0.123$, $d = 0.61$), θ ($t_{40} = 1.60$, $p = 0.189$, $d = 0.50$), β2 ($t_{40} = 0.68$, $p = 0.096$, $d = 0.21$) frequency bands. In the vertex-based comparison, patients with GGE had higher power than siblings in all frequency bands but θ (figure 4C), mainly in occipital regions. Global power in siblings was higher than in controls (figure 4A) in β1 ($t_{60} = 2.53$, $p = 0.044$, $d = 0.71$) and β2 bands ($t_{60} = 2.62$, $p = 0.044$, $d = 0.73$), but not in the remaining frequency bands (δ: $t_{60} = 0.16$, $p = 0.191$, $d = 0.04$; θ: $t_{60} = 1.28$, $p = 0.108$, $d = 0.36$; α: $t_{60} = 0.85$, $p = 0.246$, $d = 0.24$; γ: $t_{60} = 1.02$, $p = 0.193$, $d = 0.29$). Differences at vertices did not reach statistical significance ($p_{FWE} > 0.05$).

Heritability of Imaging Patterns

ICC values, which represent heritability estimations of MEG-derived patterns, strongly correlated with β1 connectivity differences of patients against controls ($r_s = 0.59$, $p = 1.88e^{-08}$) and negatively to β2 levels ($r_s = -0.39$, $p = 4.23e^{-04}$). The correlation of ICC and connectivity differences in the remaining frequency bands did not reach significance (δ: $r_s = -0.01$, $p = 0.900$; θ: $r_s = -0.10$, $p = 0.364$; α: $r_s = 0.14$, $p = 0.223$; γ: $r_s = 0.18$, $p = 0.120$). ICC values for power were significantly associated with GGE contrast maps in all frequency bands except α and γ (δ: $r_s = 0.60$, $p = 8.6e^{-09}$; θ: $r_s = 0.46$, $p = 2.5e^{-05}$; α: $r_s = 0.18$, $p = 0.109$; β1: $r_s = 0.59$, $p = 1.47e^{-08}$; β2: $r_s = 0.34$, $p = 0.002$; γ: $r_s = 0.15$ $p = 0.17$). In the following, we only report results for ICC maps with positive and significant correlations with the GGE phenotype ($p < 0.05$), implying genetic contribution to disease-related patterns (figures 1B and 2B). ICC values for connectivity in β1 frequency band were highest in rostral and caudal anterior cingulate, orbitofrontal, paracentral, entorhinal, and inferior parietal regions (ICC > 0.4) and lower in temporal regions and subcortical nuclei (ICC > 0.2) figure 1B). ICC estimates were generally higher for power than connectivity and peaked

Figure 2 Power Within Families Related to Genetic Generalized Epilepsy (GGE) Imaging Patterns



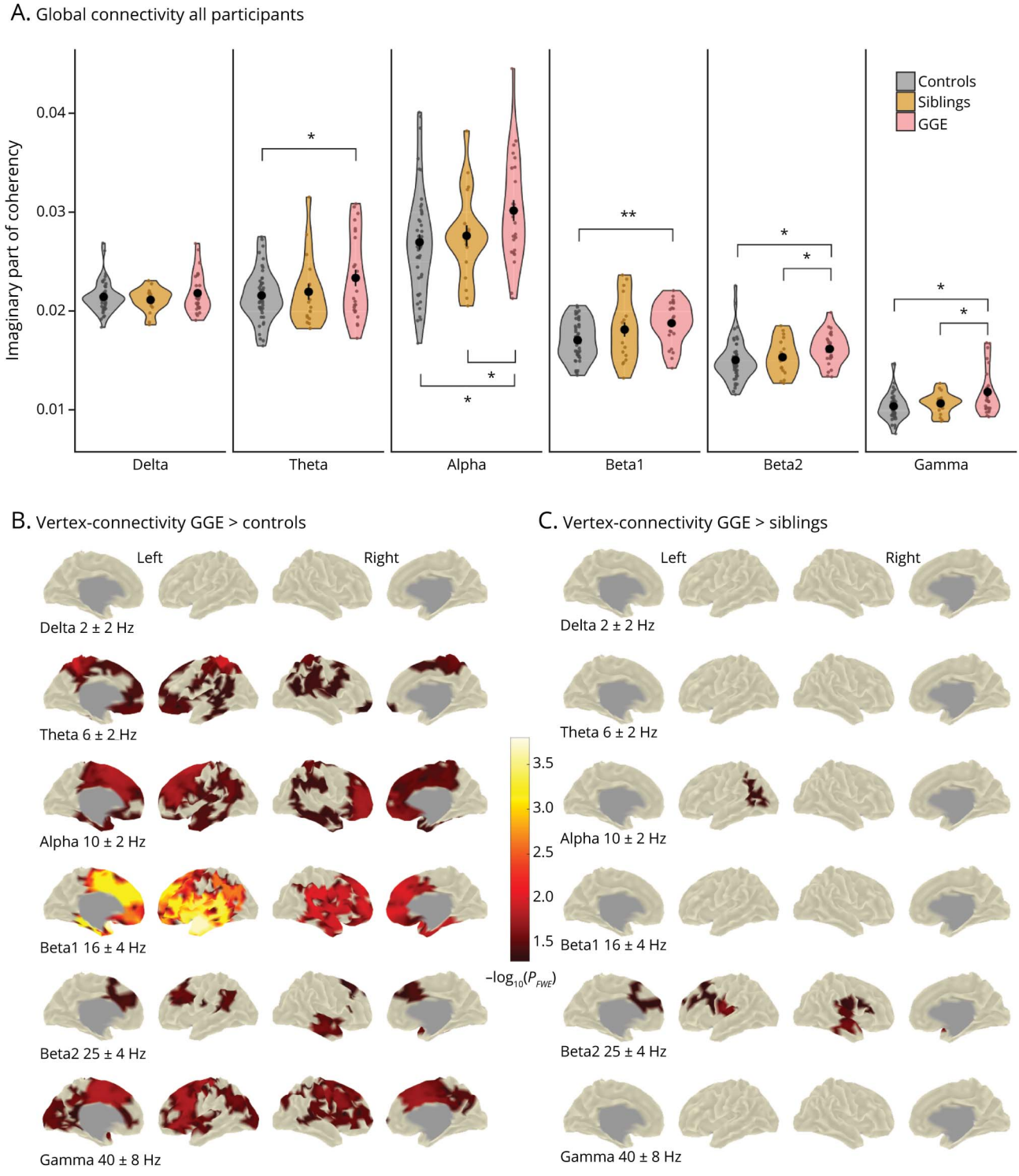
(A) Individual global power values of patients with GGE and siblings are plotted with regard to their family membership (columns of data within frequency bands). Data of patients with GGE without a corresponding sibling are not shown. GGE syndromes of patients in each family are indicated on the x-axis. Childhood absence epilepsy and juvenile absence epilepsy are referred to absence epilepsies (AE). For visualization purposes, power data were log10-transformed. (B) Color-coded heritability estimates (intraclass correlation [ICC] values) per region based on the Desikan-Kiliany atlas[26] with small to large group-level differences between patients with GGE and controls (Cohen $d > 0.2$). Only ICC maps with a positive and significant correlation with averaged effect sizes are shown ($p < 0.05$). The color coding indicates the strength of ICC values in those regions for power differences. ICC estimates were derived from random effect components of mixed models for each region, taking group and age effects into account. A large ICC indicates correlated imaging patterns for patient–sibling pairs in a family (n = 14) and thus heritability of the metrics. Cortical regions are displayed in the left column and subcortical regions are shown separately in the right column of the plot. GTCS = generalized tonic-clonic seizures; JME = juvenile myoclonic epilepsy.

in β frequency bands in temporal, subcortical, and parietal regions such as lingual gyrus and cuneus as well as postcentral gyrus (ICC > 0.5). ICC maps for δ and θ frequency bands showed similar patterns but generally lower ICC values (ICCs up to ~0.5) (figure 2B).

Clinical Variables and Imaging Findings

We carried out secondary analyses to evaluate the relation of clinical variables with brain oscillations. We investigated whether networks of patients with GGE with GSWD during the MEG recordings differ from patients without GSWD. Although trials containing GSWD (±10 seconds of data) were rejected in all analyses, patients with GSWD in the recording had higher connectivity in the δ frequency band (global: $t_{22} = 2.95$, $p = 0.004$, $d = 1.26$; vertex-based: $p < 0.05$; figure 5A). There was also a tendency of higher power across frequency bands in those patients, with significant differences for δ (global: $t_{22} = 2.55$, $p = 0.010$, $d = 1.09$; vertex-based: $p < 0.05$) and β1 frequency bands (global: $t_{22} = 2.23$, $p = 0.019$, $d = 0.96$; vertex-based: $p < 0.05$; figure 5A). Mean global power and connectivity for patients without GSWD during the recording remained higher for patients than controls and never dropped below the mean levels of siblings (data not shown). Patients taking 2 or more drugs at the study date had lower

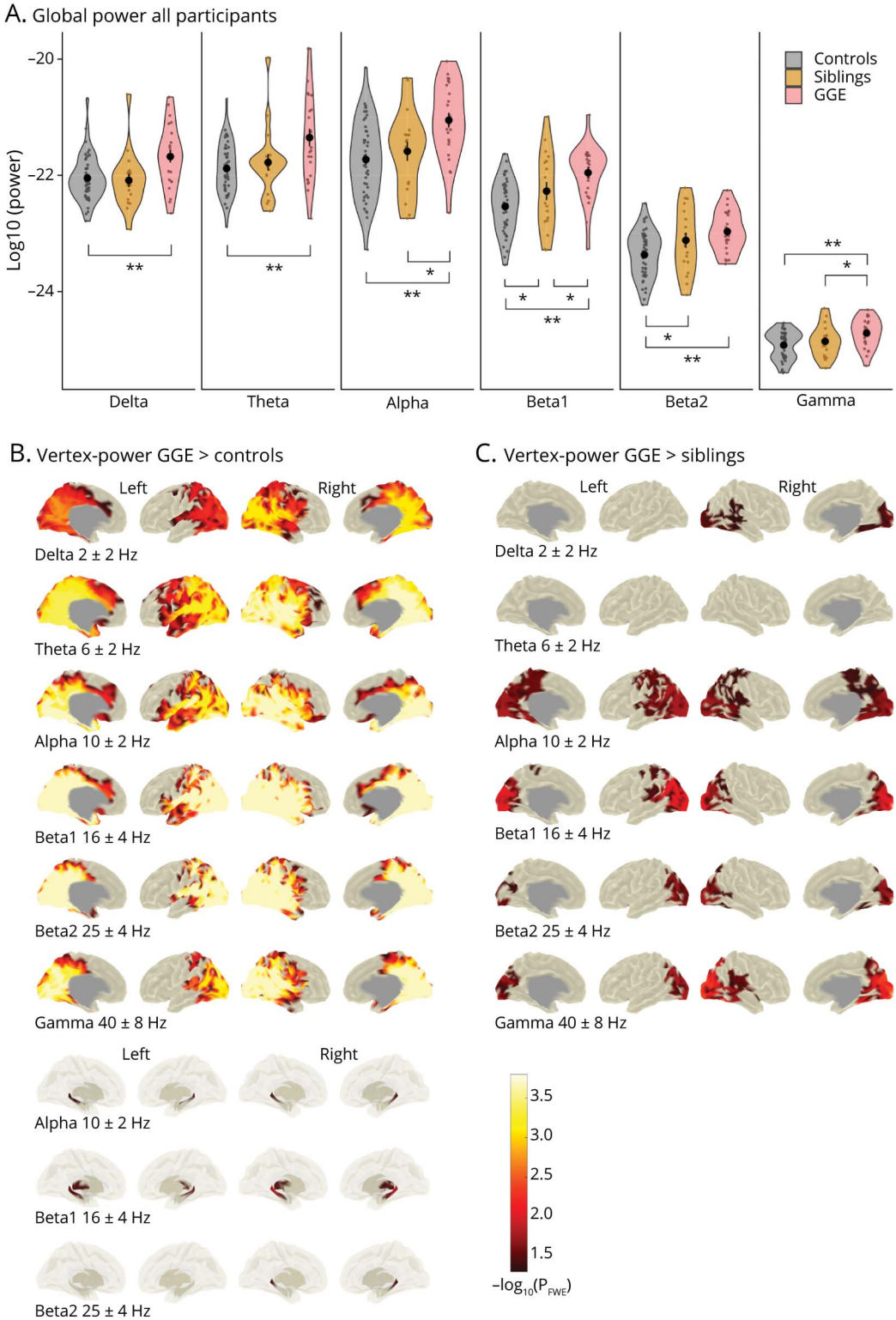
Figure 3 Group-Level Connectivity Differences



(A) Violin plots show individual data points, the density of the data, group means, and standard errors of the means for the global imaginary part of coherency in each frequency band (controls, n = 45; siblings, n = 18; patients with genetic generalized epilepsy [GGE], n = 25). Asterisks denote statistical significance at *p < 0.05 and **p < 0.001 for permutation-based group comparisons. (B) The plot highlights vertices with significantly higher connectivity values in patients with GGE (n = 25) than in controls (n = 45) and (C) higher connectivity values in patients with GGE (n = 25) than in siblings (n = 18). The color scale indicates –log10 p with a cutoff of 1.3 (corresponding to p < 0.05, familywise error corrected). In all analyses, age was included as covariate of no interest.

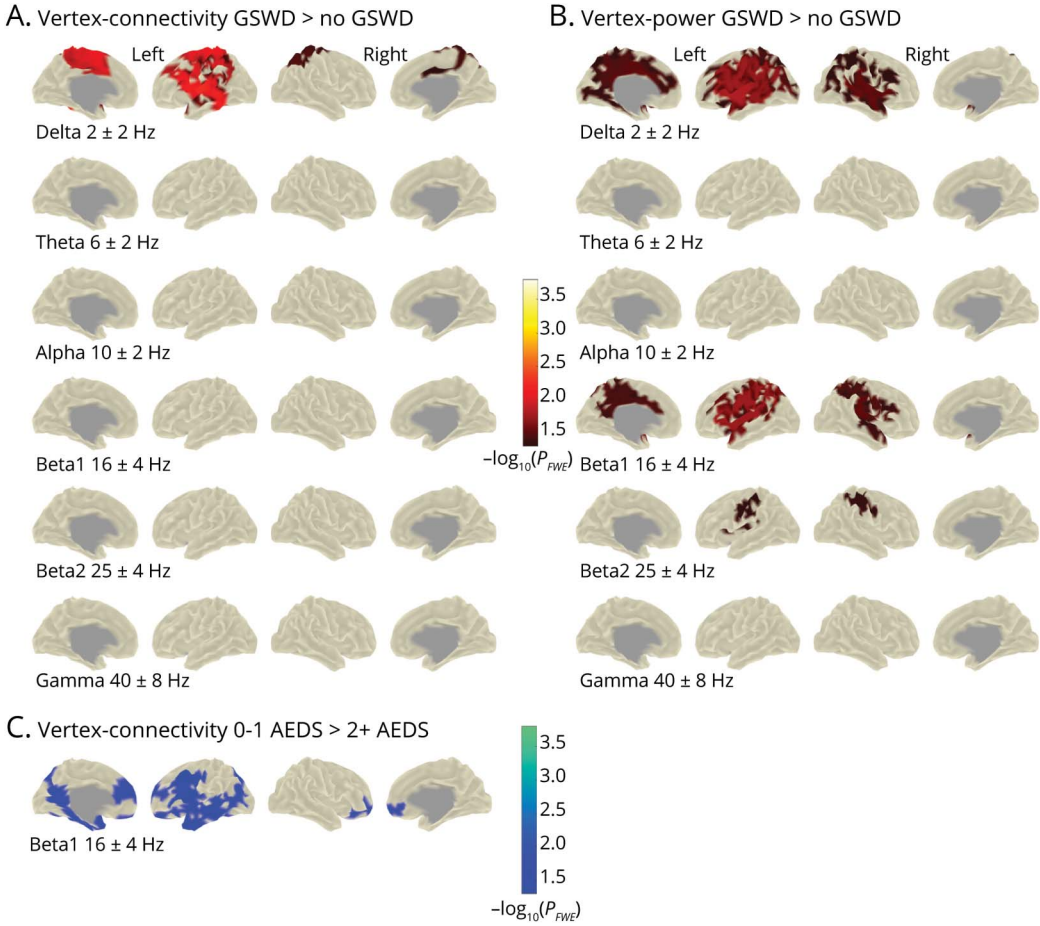
connectivity in β1 frequency band than patients taking less than 2 drugs (global: $t_{21} = -2.04$, $p = 0.029$, $d = -0.98$; vertex-based: $p < 0.05$, figure 5C). The effect was stronger when accounting for the presence of GSWD during MEG recordings. There were no differences for power in any of the frequency bands studied (all $p > 0.05$). Other clinical factors such

Figure 4 Group-Level Power Differences



(A) Violin plots show individual data points, the density of the data, group means, and standard errors of the means for global power in each frequency band (controls, n = 45; siblings, n = 18; patients with genetic generalized epilepsy [GGE], n = 25). Asterisks denote statistical significance at $*p < 0.05$ and $**p < 0.001$ for permutation-based group comparisons. For visualization purposes, power data were log10-transformed. (B) The plot highlights cortical and subcortical vertices with significantly higher power values in patients with GGE (n = 25) than in controls (n = 45). (C) The plot shows vertices with significantly higher power values in patients with GGE (n = 25) than in siblings (n = 18). The color scale indicates −log10 p with a cutoff of 1.3 (corresponding to $p < 0.05$, familywise error corrected). In all analyses, age was included as covariate of no interest.

Figure 5 Clinical Variables and Imaging Metrics



Vertex plots highlight cortical vertices with higher (A) connectivity and (B) power values in patients with genetic generalized epilepsy (GGE) with generalized spike-wave discharges (GSWD) (n = 9) than patients without GSWD (n = 16) during the magnetoencephalography recordings. These effects were present after exclusion of trials containing GSWD ±10 seconds of data and corrected for age effects. (C) The plot shows significantly lower connectivity in patients with GGE taking 2 or more antiepileptic drugs (n = 6) than patients taking fewer than 2 drugs (n = 19) at the study date. Age and presence of GSWD was included as covariate of no interest. Color scales indicate –log10 *p* with a cutoff of 1.3 (corresponding to $p < 0.05$, familywise error corrected).

as age at onset, disease duration, or seizure control were not associated with global and vertex-based connectivity or power (all $p > 0.05$).

Discussion

We assessed power and phase-based connectivity during rest in patients with GGE and their healthy siblings to evaluate endophenotypic potential of electrophysiologic metrics. Patients with GGE showed bilateral, widespread, and highly increased power and connectivity compared with controls. Asymptomatic siblings presented with intermediate levels between patients and controls, particularly in β frequencies, suggesting genetic background as major driver for those patterns.

We expanded and replicated previous work of ours using MEG data of mixed GGE cohorts,[15,16] confirming strong interictal network differences in GGE as quantified with the investigated imaging metrics. Both global and local measures have been reliable in source-space analyses using the same processing pipeline.[20] Increased power in GGE is a previously reported finding,[27,28] but M/EEG connectivity studies on source level are scarce.[15,16] High β band connectivity in GGE was the most consistent finding across the studies, followed by increases in θ, α, and γ bands. Epileptic seizures commonly involve pathologic synchronization.[29] Also during the interictal state, patients have had higher liability to synchronize.[30,31]

Increased network connectivity and power was also observed in siblings without active epilepsy.[6] Intraclass correlations substantiate heritability in regions, where patients with GGE strongly differed from controls, specifically for β1 band connectivity and both β1 and β2 power. In δ and θ power, heritability estimates corresponded to the phenotype of patients, but effect sizes and ICC values were comparably low. Increased β2 connectivity patterns in patients compared with controls were less concordant within families.

Elevated MEG connectivity was mostly heritable in anterior cingulum, orbitofrontal, and superior frontal regions. In GGE, direct evidence for a genetically determined functional dysregulation in frontal cortex is limited, but has been documented for prefrontal and cingulate morphology.[10] Altered structural network integration of the frontal cortex[10] potentially leads to cognitive impairments in patients with JME and similarly in their healthy siblings.[7-10] Furthermore, network power and connectivity was heritable in central brain regions. Hyperactivations in motor systems have been suggested as endophenotypes of JME during cognitive fMRI,[13,14] but also for a mixed GGE cohort during resting-state.[30] We complement these findings by studying much faster neuronal oscillations using MEG and without vascular confounds. Our results point to more globally increased network levels in patients with GGE and siblings, irrespective of a task involved. This includes increased power patterns in mesiotemporal cortices and in subcortical structures with a strong genetic substrate. ICC estimates reached levels of up to 0.85, particularly in β frequencies, indicating that familial and, thus, likely genetic factors explain the majority of the observed variance. Thalamocortical circuits are critical for GGE[32] and structural alterations in the thalamus[33,34] have been linked to subcortico-cortico hub organization in GGE.[33] Using MEG, it is debatable whether signals of deep brain structures can be captured. Yet recent work has used simultaneous intracerebral and MEG recordings in patients with epilepsy and demonstrated detectability of signals generated in mesial temporal lobe structures as well as thalamic activity at the surface.[35] In line with our results, mesiotemporal task-based activations cosegregated in patients with JME and their healthy siblings, along with changes in hippocampal morphology.[11] The role of increased occipital power in our GGE cohort is less clear. Low-density EEG data[28] and microstructural alterations in fronto-occipital white matter association tracts[36] point to the involvement of occipital areas in GGE. In our study, patients and siblings mostly differed in occipital power, suggesting that this finding may not be due to genetic factors but to other unknown disease-related effects. Overall, we consolidate findings of earlier GGE–sibling studies using (f)MRI and add evidence for resting-state trait heritability in extended brain networks at higher temporal resolution.

Electrophysiologic studies in twins suggest strong genetic influence on brain oscillations relative to environmental factors, particularly for wideband power ($h^2 \sim 0.5$–0.8).[18,37] Heritability for connectivity on the cortical source level has been lower than for power and highest in α and β frequencies (10%–20%).[19] Lower reproducibility of connectivity metrics may, at least partly, explain a lower heritability estimate compared to power. Similarly, connectivity in α and β bands has been more repeatable than in other frequency bands.[20] Network alterations characterizing GGE were captured in brain rhythms of different frequencies, but imaging phenotypes of siblings and patients with GGE most strongly correlated in β frequencies. β band oscillations are classically related to sensory and motor processing[38] as well as long-distance synchronization.[39] GABAergic processes are presumably involved in the generation of MEG β oscillations as shown with endogenous GABA concentrations in humans.[40] GABAA receptor gene variants constitute GGE disease risk[5,41] and one of those candidates, the *GABRA2* gene, has been linked to EEG β band activity.[42,43] Moreover, recently discovered genetic markers in GGE have been enriched in the frontal cortex, specifically in the dorso-lateral prefrontal cortex.[5] Genetic signals have further converged on the inferior temporal lobe, angular gyrus, cingulum, and subcortical tissue, but less strongly.[5] Our ICC maps show substantial spatial correspondence to those findings, emphasizing the functional relevance of imaging resting-state markers as investigated in our study. Previously, oscillatory activity has been successfully used to detect psychiatric liability genes[43] and EEG coherence has served as an endophenotype for alcohol use disorders.[44] Yet the functional role of spectral perturbations is not fully understood. Here, we can only speculate about molecular changes occurring within specific networks in the brain, such as an excitation–inhibition imbalance, as a putative key factor in epilepsy. Through coupling mechanisms, the coordination of neuronal spike timing might be affected across networks, in sum leading to increased oscillatory amplitudes.[45]

Disease duration and age at onset did not correlate with the imaging patterns in our GGE cohort. These clinical variables do not necessarily describe disease severity and the lack of a significant association with the imaging findings may support the notion of a genetic imaging trait. Patients showing epileptic discharges during the MEG recording had higher δ connectivity and δ and β power increases at rest (after careful exclusion of segments with GSWD ± 10 seconds of data). These patterns had a spatial profile with a temporal and central focus. GSWD typically have a frequency around 3 Hz and it is possible that we captured network dynamics around the onset or offset, evolving during a considerable time span.[31] Only 9 patients in our study showed GSWD and at least 5 of them had experienced seizures within the past year. However, seizure control was not associated with significantly different network patterns in our cohort and might not be a sensitive marker in a rather well-controlled cohort. Similarly, persistent GSWD do not necessarily have an effect on long-term seizure prognosis.[46] Moreover, patients with higher medication load had lower connectivity in the β1 frequency band. Because only 6 patients received more than 2 antiepileptic agents at the time of the study, we can only speculate about a network downregulation through antiepileptic treatment. Normalizing effects of antiepileptic drugs on background synchronization have been demonstrated before.[47] Further investigations are needed to confirm our exploratory results and study drug-specific effects.

This study has limitations. We found alterations for both power and imaginary part of coherency, a phase-based metric. Power and phase characterize different aspects of neural signals; however, both physiologic and nonphysiologic coupling between those characteristics has been noted.[45] In particular, high values in phase-based connectivity require a temporally stable phase relationship of signals, which depends on the

SNR and eventually on signal power. In our study, the topography for GGE power differences had a posterior focus and was distinct from connectivity maps with a more frontal emphasis. Recent work suggests that spontaneous MEG networks can be decoupled into anterior and posterior states, both connected to the posterior cingulate cortex, likely reflecting functional specialization.[48] Given the different effects of power and connectivity on the MEG signal topography, it is very likely that both measures captured independent features. Furthermore, we cannot assess the mechanisms of genetic control over oscillatory markers. Methodologic aspects may also affect heritability estimates. For example, higher sensitivity of connectivity measures to noise might be a reason for generally lower effect sizes than for power and weaker ICC values in our study. Accordingly, higher statistical power is needed for significant connectivity differences between siblings and controls or patients, given the intermediate levels of the siblings. The relatively small number of 14 families that were available for the study also limits the precise estimation of the ICCs. Finally, we cannot discern specific effects for the GGE subsyndromes due to the small sample size. Yet multiple GGE subtypes occur within the same families[49] and overlap of genetic risk factors has been suggested.[5] Network phenotypes as assessed in our study could reflect shared pathophysiologic features across the syndromes, such as the occurrence of GSWD. Interestingly, GSWD have been more frequently observed in unaffected first-degree relatives of patients with GGE than in the general population,[50] which again points to genetic contributions to network function in GGE.

We propose that increased interictal MEG power and connectivity in frontocentral and temporo-parietal cortical regions are a hallmark of GGE. These network features are likely driven by genetic factors and not by the presence or absence of the active disease or clinical confounds. Siblings without epilepsy had similarly increased network levels during rest, predominantly in β frequencies. We show that power and phase-based connectivity are heritable and may serve as markers to link imaging with genetics in epilepsy.

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Disclosure

N.K. Focke received speaker bureau and consultancy fees from Bial, UCB, Eisai, and EGI/Phillips, all unrelated to the present work. H. L. received speaker bureau and consultancy fees from Arvelle, Bial, Biomarin, Eisai and UCB, all unrelated to the present work. C. Stier, A. Elshahabi, Y. Li Hegner, R. Kotikalapudi, J. Marquetand, and C. Braun report no disclosures. Go to Neurology.org/N for full disclosures.

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Appendix Authors

Name	Location	Contribution
Christina Stier, MSc	Clinic of Clinical Neurophysiology, University Medical Center Göttingen; and Department of Neurology and Epileptology, Hertie Institute of Clinical Brain Research, University of Tübingen, Germany	Designed and conceptualized the study, major role in the acquisition of data, analyzed the data, performed statistical analyses, interpreted the results, drafted the manuscript
Adham Elshahabi, MD, MSc	Department of Neurology and Epileptology, Hertie Institute of Clinical Brain Research, University of Tübingen, Germany; and Department of Neurology, University Hospital Zurich, Switzerland	Major role in the acquisition of data, revised the manuscript for intellectual content
Yiwen Li Hegner, MD, PhD	Department of Neurology and Epileptology, Hertie Institute of Clinical Brain Research, University of Tübingen, Germany	Provided critical input on the methods, revised the manuscript for intellectual content
Raviteja Kotikalapudi, PhD	Clinic of Clinical Neurophysiology, University Medical Center Göttingen; Department of Neurology and Epileptology, Hertie Institute of Clinical Brain Research, University of Tübingen, Germany; and Institute of Psychology, University of Bern, Switzerland	Major role in the acquisition of data, revised the manuscript for intellectual content
Justus Marquetand, MD	Department of Neurology and Epileptology, Hertie Institute of Clinical Brain Research, University of Tübingen, Germany	Major role in the acquisition of data, revised the manuscript for intellectual content
Christoph Braun, PhD	MEG-Center, University of Tübingen, Germany; CIMeC, Center for Mind/Brain Sciences, University of Trento, Italy; and Department of Neurology and Epileptology, Hertie Institute of Clinical Brain Research, University of Tübingen, Germany	Provided critical input on the methods, project supervision, revised the manuscript for intellectual content

Appendix *(continued)*

Name	Location	Contribution
Holger Lerche, MD	Department of Neurology and Epileptology, Hertie Institute of Clinical Brain Research, University of Tübingen, Germany	Revised the manuscript for intellectual content
Niels K. Focke, MD	Clinic of Clinical Neurophysiology, University Medical Center Göttingen; and Department of Neurology and Epileptology, Hertie Institute of Clinical Brain Research, University of Tübingen, Germany	Designed and conceptualized the study, project supervision, interpreted the results, revised the manuscript for intellectual content

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Heritability of Magnetoencephalography Phenotypes Among Patients With Genetic Generalized Epilepsy and Their Siblings

Christina Stier, Adham Elshahabi, Yiwen Li Hegner, et al.

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4.2 COMBINED ELECTROPHYSIOLOGICAL AND MORPHOLOGICAL PHENOTYPES IN PATIENTS WITH GENETIC GENERALIZED EPILEPSY AND THEIR HEALTHY SIBLINGS

Combined electrophysiological and morphological phenotypes in patients with genetic generalized epilepsy and their healthy siblings

Christina Stier[1, 2], Markus Loose[1], Raviteja Kotikalapudi[1, 2, 3], Adham Elshahabi[2,4], Yiwen Li Hegner[2], Justus Marquetand[2, 5], Christoph Braun[2, 6, 7], Holger Lerche[2], Niels K Focke[1, 2]

[1] *Clinic of Neurology, University Medical Center Göttingen, Göttingen, Germany*
[2] *Department of Neurology and Epileptology, Hertie Institute for Clinical Brain Research, University of Tübingen, Tübingen, Germany*
[3] *Institute of Psychology, University of Bern, Bern, Switzerland*
[4] *Department of Neurology, University Hospital Zurich, Zurich, Switzerland*
[5] *Department of Neural Dynamics and Magnetoencephalography, Hertie-Institute for Clinical Brain Research, University of Tübingen, Tübingen, Germany*
[6] *MEG-Center, University of Tübingen, Tübingen, Germany*
[7] *CIMeC, Center for Mind/Brain Sciences, University of Trento, Rovereto, Italy*

Corresponding authors

Prof. Dr. med. Niels Focke
Universitätsmedizin Göttingen
Klinik für Neurologie
Robert-Koch-Str. 40
37075 Göttingen
Email: niels.focke@med.uni-goettingen.de
Phone: +49 (0) 551 39 68455

Christina Stier, M. Sc.
Universitätsmedizin Göttingen
Klinik für Neurologie
Robert-Koch-Str. 40
37075 Göttingen
Email: christina.stier@med.uni-goettingen.de
Phone: +49 (0) 551 39 65106

Abbreviations

AED = antiepileptic drugs
FWE = family-wise error
GGE = genetic generalized epilepsy
GSWD = generalized spike-wave discharges
(HD)-EEG = (high-density) electroencephalography
JME = juvenile myoclonic epilepsy
MEG = magnetoencephalography
MRI = magnetic resonance imaging

Abstract

Objective

Genetic generalized epilepsy is characterized by aberrant neuronal dynamics and subtle structural alterations. We evaluated whether a combination of magnetic and electrical neuronal signals and cortical thickness would provide complementary information about network pathology in GGE. We also investigated if these imaging phenotypes were present in healthy siblings of the patients to test for genetic influence.

Methods

In this prospective, cross-sectional study, we analyzed five minutes of resting-state data acquired using electroencephalography (EEG) and magnetoencephalography (MEG) in patients, their siblings, and controls, matched for age and sex. We computed source-reconstructed power and connectivity in six frequency bands (1-40 Hz) and cortical thickness (derived from MRI). Group differences were assessed using permutation analysis of linear models for each modality separately and jointly for all modalities using a non-parametric combination.

Results

Patients with GGE (n = 23) had higher power than controls (n = 35) in all frequencies, with a more posterior focus in MEG than EEG. Connectivity was also increased, particularly in frontotemporal and central regions in theta (strongest in EEG) and low beta frequencies (strongest in MEG), which was eminent in the joint EEG/MEG analysis. EEG showed weaker connectivity differences in higher frequencies, possibly related to drug effects. The inclusion of cortical thickness reinforced group differences in connectivity and power. Siblings (n = 18) had functional and structural patterns intermediate between those of patients and controls.

Significance

EEG detected increased connectivity and power in GGE similar to MEG, but with different spectral sensitivity, highlighting the importance of theta and beta oscillations. Cortical thickness reductions in GGE corresponded to functional imaging patterns. Our multimodal approach extends the understanding of the resting state in GGE and points to genetic underpinnings of the imaging markers studied, providing new insights into the causes and consequences of epilepsy.

Keywords

Resting-state, oscillations, cortical thickness, interictal, endophenotypes

Introduction

Genetic generalized epilepsy (GGE) is a common epilepsy syndrome with polygenic etiology.[1] Rapid neuronal changes such as generalized spike-wave discharges (GSWD) or generalized seizures are a hallmark of GGE. However, the link between the genetic pathology underlying the disease and its systemic effects on macro-scale brain dynamics is not well understood. Using MEG, we have previously shown that increased resting-state power and network synchronization are characteristic of GGE and are similarly present in healthy siblings of the patients.[2] We hypothesized that this could also be observed with EEG, which is generally more available than MEG in routine clinical practice. In principle, MEG and EEG signals reflect the same neuronal sources. However, due to the different sensitivity profiles of the techniques, one can expect that the recording of both signals could also reveal complementary information.[3] For GGE, few studies have exploited the benefit of employing both EEG and MEG together, and most have focused on localizing the source of GSWD.[4, 5] Using both techniques in the same individuals at rest could therefore provide additional information about aberrant networks in GGE. In addition to functional alterations in GGE, subtle structural changes such as cortical thinning are known.[6] However, the relationship between those changes and fast oscillatory neuronal activity in GGE has not been investigated in detail. Atrophic patterns could result from disease progression or disease activtiy,[7] but the evidence is inconclusive[8], and longitudinal studies are lacking. Cortical thickness reflects cell density and cytoarchitecture, among other factors, and is highly heritable. [9] Consequently, microstructural changes in GGE may be genetically driven and linked to electrophysiological alterations. If so, the statistical combination of all three modalities, that is, EEG, MEG, and MRI in the same cohort, should point to common network alterations. Together with attempts to understand the heritability of these states, this could lead to improved diagnosis and prognosis for GGE in the future. GGE markers that are heritable, so-called endophenotypes[10], are thought to reflect causative disease mechanisms rather than clinical presentation. Some (f)MRI research has demonstrated such traits of GGE subtypes,[7, 11-15] including increased activations of the motor system[12-15], aberrant cortical folding and surface[7] and hippocampal structure and function.[11] So far, there is little evidence of such endophenotypic markers at higher temporal resolution[16] and in mixed GGE types.

Here, we adopted a multimodal approach that integrated structural and functional features of GGE to promote a more holistic understanding of GGE network pathology and its genetic basis. In a first step, we compared EEG resting-state measurements to MEG measurements in the same cohort reported earlier.[2] We then integrated both modalities in a unified statistical analysis and explored the correspondence between cortical thickness and functional group maps. Finally, we examined whether the functional and structural changes could be genetically determined by studying healthy siblings of the patients.

Methods

Participants

We studied 28 consecutive patients, 21 healthy siblings and 50 controls, who were recruited through the Department of Neurology, University Hospital of Tübingen, Germany, and in the local area. Patients were diagnosed with GGE according to the International League Against Epilepsy.[17] Siblings and controls were free of any neurologic or psychiatric disorders, had never experienced seizures, and did not take any medication at the time of the measurements. After exclusion of data because of technical problems, artifacts, or sleep during recordings, 23 patients, 18 siblings (related to 13 patients), and 35 controls were available for further analysis. All MRI scans were visually rated as normal except for three non-specific findings (two patients with uncomplicated cysts and one patient with a non-specific white matter lesion). The groups were comparable for sex (female patients: 61%; siblings: 50%; controls: 51%; $X^2 = 0.5$, $p = 0.78$) and age (median (IQR): patients: 26 (22-40); siblings: 26 (22-42); controls: 25 (22-35); $F = 0.1$, $p = 0.91$). Five of the 23 patients were diagnosed with juvenile absence epilepsy, four patients with childhood absence epilepsy, five patients with juvenile myoclonic epilepsy (JME), four patients with isolated generalized tonic-clonic seizures and five patients could not be further classified. All but two patients were on antiepileptic medication (AED; mean number of drugs: 1.2, range: 0-3). For more clinical details, see Table S1. Study approval was received from the local Ethics Committee of the Medical Faculty of the University of Tübingen. The study was conducted in compliance with the principles of the Declaration of Helsinki. All individuals gave informed consent to participate in the study.

EEG/MEG recordings

The individuals were measured in a supine position in the MEG center of the University of Tübingen, using a 275-channel MEG system (CTF Inc., Vancouver, Canada) and subsequently using a 256-channel EEG system (GES400; EGI, Inc./Philips-Neuro, Eugene). We continuously recorded 30 minutes of resting-state eyes-closed each (sampling rate EEG: 1 kHz; MEG: 568 Hz) and instructed the individuals to relax, not to fall asleep, and not to think of anything in particular. This rather long acquisition time was chosen to obtain sufficient data after exclusion of segments with GSWD.

MR image acquisition

All individuals underwent MRI scanning either on a Siemens Magnetom Trio 3T scanner equipped with a 12-channel head coil (10/35 controls, 4/23 patients) or on the Siemens Magnetom Prisma 3T system (Siemens, AG, Erlangen, Germany) with a 64-channel head coil (25/35 controls, 18/18 siblings, 19/23 patients). Sagittal high-resolution T1-weighted images were acquired (3D-MPRAGE, repetition time = 2.3 s, echo time = 3.03 ms, flip angle = 8°, voxel size = 1 x 1 x 1 mm).

Surface based mapping

FreeSurfer 6.0.0 (https://surfer.nmr.mgh.harvard.edu/) was used to reconstruct individual cortical surfaces sampled at the pial and the grey-white boundary ('smoothwm'). To ensure anatomical correspondence among individuals and modalities, we applied SUMA[18] to recreate each surface (density factor ld = 10) based on a FreeSurfer standard template ('fsaverage'). This procedure yielded 1002 common vertices per hemisphere for cortical thickness estimations and as EEG/MEG source-points, allowing vertex-based group contrasting.

EEG/MEG head models

In order to conduct source-level analyses, we built volume conduction models based on individual cortical meshes yielded by the SUMA procedure and SPM12 (https://www.fil.ion.ucl.ac.uk/spm/software/spm12/) brain segmentations. The EEG electrodes were aligned with the anatomical landmarks (nasion, preauricular points) and projected onto the scalp mesh. For MEG, cortical meshes were realigned to the CTF sensor space using fiducial positions marked in the MR image and reference coils placed during the measurements. Leadfields were computed based on either a three-layer boundary element model for EEG or a single-shell model for MEG using Fieldtrip[19] in Matlab (version 9.0, R2016a, Mathworks Inc.). More technical details and references can be found elsewhere.[20]

Data processing and source localization

EEG and MEG data were separately processed using Fieldtrip.[19] First, we applied a first-order Butterworth band-pass filter (1-70 Hz) and a band-stop filter to remove line noise (at 50, 100, and 150 Hz). Data were downsampled (150 Hz) and segmented into trials of 10 s length. Each trial was visually inspected and rejected if noisy (e.g. muscle artifacts, sensor jumps). We also excluded trials with GSWD plus trials preceding and following the event, respectively (±10 seconds). Cardiac artifacts and eye movements were extracted by independent component analyses and manually rejected. In a second review, we scored vigilance of the individuals according to the criteria of the American Academy of Sleep Medicine (https://aasm.org/). Only trials rated as awake were further considered. Thirty trials were randomly selected for source analysis because previous work has shown good reliability for the metrics of interest for 5 min data. [20]

Spectral analyses were performed using fast Fourier time-frequency transforms and multitapers for six frequency bands (delta: 2 ± 2 Hz, theta: 6 ± 2 Hz, alpha 10 ± 2 Hz, beta1 16 ± 4 Hz, beta2 25 ± 4 Hz and gamma 40 ± 8 Hz). Power and cross-spectral densities were estimated on the Fourier transformed sensor data and then projected to the source space using beamforming[21] in each frequency band. Power was calculated for each vertex and the coherency coefficient between all pairs of vertices (n = 2004). We derived the absolute imaginary part of coherency as our connectivity measure quantifying phase synchrony between signals less affected by potential field spread.[22] To determine the total connectivity for each vertex, we averaged the strength of all its connections. We also computed a global power and connectivity value for each participant by averaging across all vertices.

Cortical thickness

Cortical thickness quantifies the distance between the gray-white matter and pial boundaries and was computed at each SUMA vertex (FreeSurfer procedure). Thickness maps were smoothed with a heat kernel of size 12 mm Full Width at Half Maximum[23] in AFNI (https://afni.nimh.nih.gov/) to account for residual spatial differences among individuals.

Joint inference

We performed joint analyses of EEG, MEG, and MRI metrics using permutation-based Non-Parametric Combination (NPC)[24] in PALM (https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/PALM). We hypothesized the existence of increased EEG and MEG connectivity and power as well as reduced cortical thickness in patients compared with controls with corresponding intermediate values in siblings. To obtain concordant directions of the modalities, we multiplied individual cortical thickness values by -1 when combining with functional data. First, one-sided *t* contrasts were separately tested for each modality based on synchronous permutations across modalities to account for dependencies. In brief, general linear models were fitted for each vertex individually, and one at the global level, with group, age, sex, total intracranial volume and scanner site as independent variables and the imaging metric as dependent variable. Second, we applied Fisher's method to combine test statistics of the modalities for each permutation. This process was repeated (number of permutations), resulting in a combined empirical distribution from which a final *p*-value was derived[25]. We used tail approximation in PALM for accelerated inference (500 permutations) and multi-level block permutation to allow sufficient exchangeability of the data, given relatedness among individuals (for details see[2]). *P*-values were family-wise error corrected (FWE) at the level of clusters resulting from threshold-free cluster enhancement (TFCE)[26] and based on the permutation distribution of the extremum statistics across all modalities. We chose a significance level of $-\log10\ p = 1.3$ (equivalent to $p < 0.05$). Cohen's *d* was calculated based on the *t* values of the group factors of the linear models and is therefore adjusted for age, sex, and intracranial volume effects. *d* describes the standardized mean difference of an effect so that $d \geq 0.8$ indicates a large, $d = 0.5$ a medium, and $d = 0.2$ a small effect.[27] In order to further explore structural alterations, we spatially remapped cortical thickness measures to 68 brain regions of the Desikan-Kiliany atlas[28] and investigated separate regional group differences using PALM.

Results

Connectivity in GGE and siblings

Patients with GGE had higher connectivity than controls, reaching significance in lower frequencies in the vertex and global EEG analysis (delta, theta, and alpha frequency bands; see Figure 2, Table S2). The EEG connectivity increases in patients were most pronounced in centrotemporal brain areas, whereas significant increases in the MEG analysis tended to have a frontotemporal focus across the entire frequency spectrum (Figure 1A and 1B) as previously reported.[2] In the joint EEG and MEG vertex analysis, the increases were strongest in theta and beta1 but also included significant patterns in the delta and alpha frequencies (Figure 1C). When combining global EEG and MEG statistics, connectivity levels in patients were significantly higher than in controls in all frequency bands studied (Table S2).

Patients did not significantly differ from siblings, but siblings tended to have higher EEG connectivity than the patients in the beta2 and gamma frequency bands (vertex-level and globally; Figure 2A and 2C, Table S2). In siblings, there was also a trend toward higher EEG connectivity compared with controls across the spectrum, but predominantly in the theta band without reaching significance (vertex-level and globally; Figure 2A and Table S2). In the MEG analysis, siblings showed higher connectivity than controls in the beta1 band, as previously reported in detail,[2] but also did not differ significantly from either the controls or the patients (vertex analysis not shown, global analysis in Table S2). See Figure S1 for effect sizes of the differences between siblings and the other groups at the vertex-level.

Combining EEG and MEG statistics did not substantially change the findings of the separate analyses in siblings.

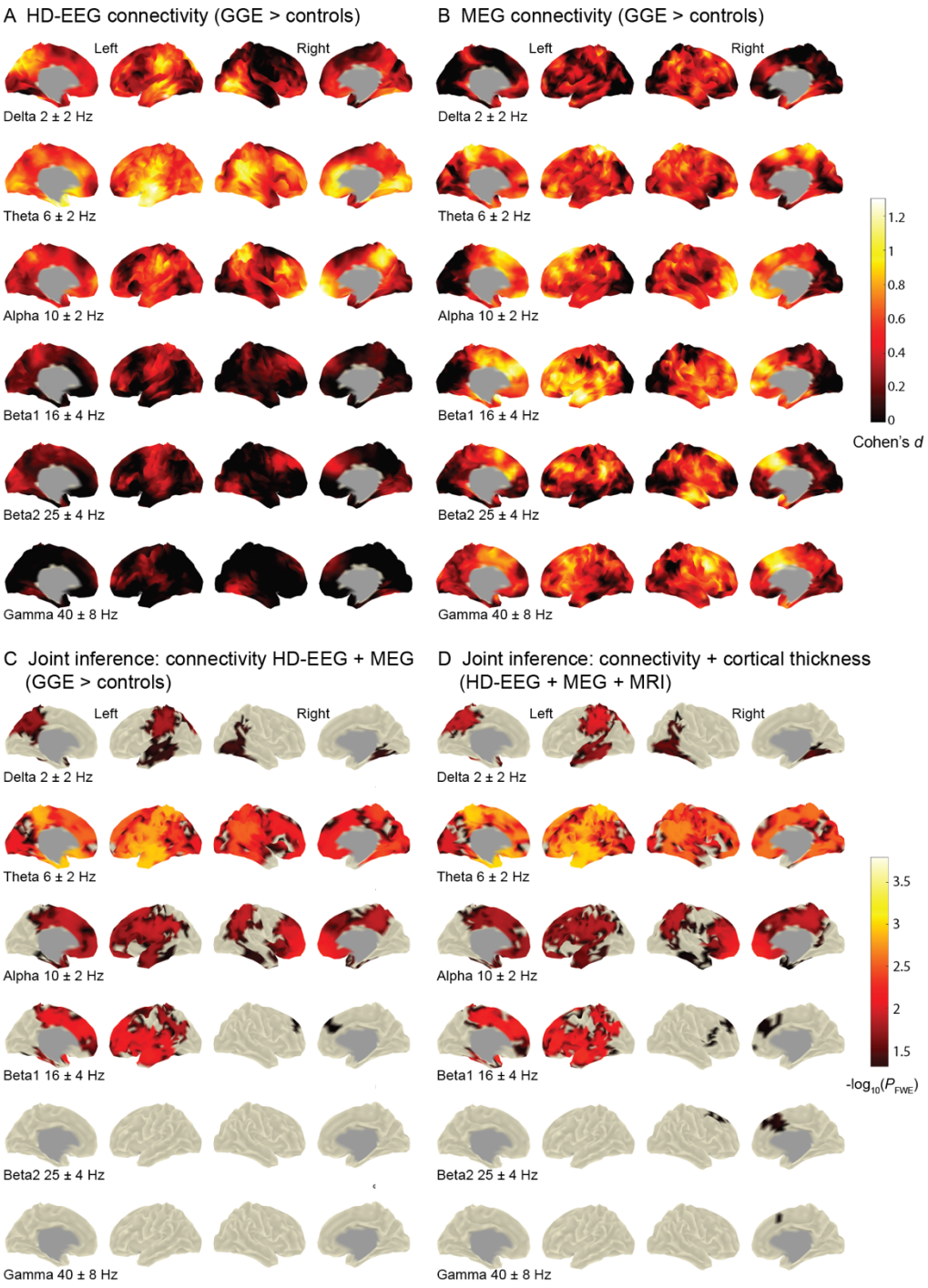

FIGURE 1 Effect sizes of separate EEG and MEG connectivity increases in GGE, and joint inference with and without cortical thickness

(A) and (B) show standardized effect sizes (Cohen's *d*) for increased connectivity in patients with GGE (n = 23) versus controls (n = 35) using EEG and MEG, respectively. Effect sizes were derived from the *t*-values of the permutation analyses of linear models. *d* is therefore adjusted for age, sex, scanner, and intracranial volume effects. *d* = 0.2 indicates a small effect, *d* = 0.5 a medium, and *d* ≥ 0.8 a large effect.[27] Note the

different spectral results of the two modalities. The EEG connectivity increases in patients were significant in the delta, theta, and alpha frequencies with a centrotemporal focus. In the MEG analysis, the increases were most pronounced in the beta1 frequency and frontotemporal areas, but reached significance in all frequency bands studied (not shown). (C) The statistical combination of EEG and MEG patterns highlighted connectivity increases, particularly in the theta and beta frequency band. (D) Inclusion of cortical thickness in the joint analysis of EEG and MEG patterns resulted in more pronounced differences between patients and controls, especially in theta and beta1 frequencies and in frontal areas in other frequency bands. We used a non-parametric combination of EEG/MEG connectivity and cortical thickness based on Fisher's method[25] with age, sex, scanner, and intracranial volume as covariates of no interest.

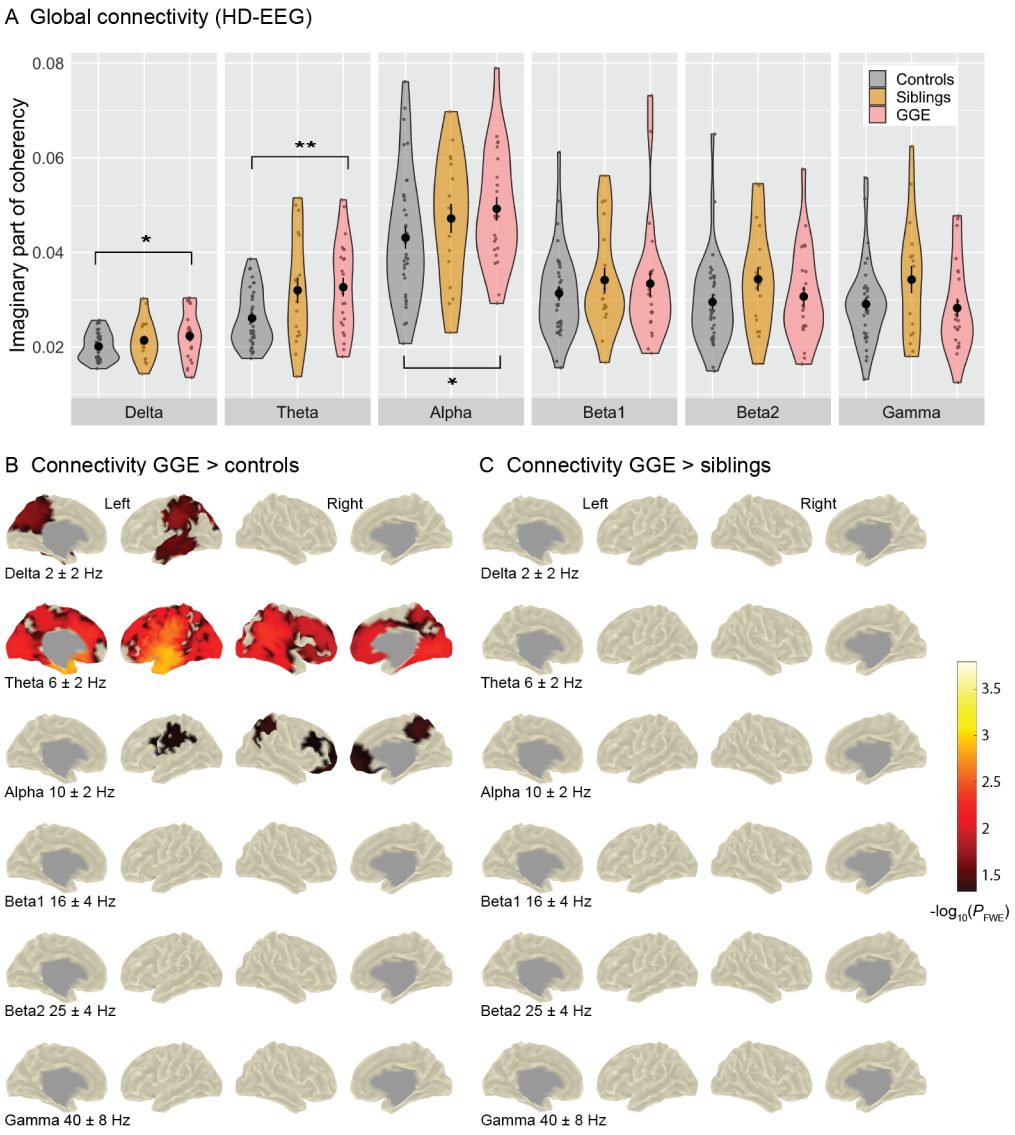

FIGURE 2 EEG analyses of global and vertex connectivity

(A) Individual global connectivity values (imaginary part of coherency) of controls (n = 35), siblings (n = 18), and patients with GGE (n = 23). Shown are the density of data, group means, and standard errors of means. Statistically significant group differences are marked with an asterisk (*$p < 0.05$, **$p < 0.001$). See Table S2 for detailed results. (B) and (C) Significantly increased vertex connectivity is highlighted in patients compared with controls, and in patients compared with siblings, respectively at a -log10 p threshold of 1.3 (equivalent to $p < 0.05$ family-wise error corrected). We used permutation-based analysis of linear models for global and vertex analyses with age, sex, scanner, and intracranial volume as covariates of no interest.

Power in GGE and siblings

EEG power was significantly higher in patients with GGE than in controls in all frequency bands studied (delta to gamma bands at vertex-level and globally, Figure 4, Table S2). A posterior focus of power increases was observed in both EEG and MEG analyses, but especially for the MEG-derived patterns in all frequency bands (Figure 3A and 3B). In the joint EEG/MEG analysis, the power increases were most pronounced in posterior regions of the brain, that is, in occipital, temporoparietal, and central regions (delta to gamma bands, Figure 3C).

Patients with GGE also had higher power than their siblings, but to a lesser extent than controls. This increased power was significant in the lower EEG frequency bands (delta to alpha at vertex-level, and globally, Figure 4A and 4C, Table S2) in the temporal-posterior regions. In the MEG analysis, power was increased occipitally and in all frequency bands except theta (not shown). In the joint vertex analyses, the power increases in patients compared with siblings were significant at all frequencies and at a global level in delta, alpha and gamma (Table S2). There were no significant EEG power differences between healthy siblings and controls, but standardized effect sizes indicated generally higher power in siblings than in controls. Similarly, MEG analysis revealed higher theta and beta power in siblings as previously shown.[2] See Figure S1 for a vertex-level and Table S2 for global comparison of effect sizes for differences between the three groups.

Combining EEG and MEG statistics did not reveal notable changes compared with separate power analyses in siblings.

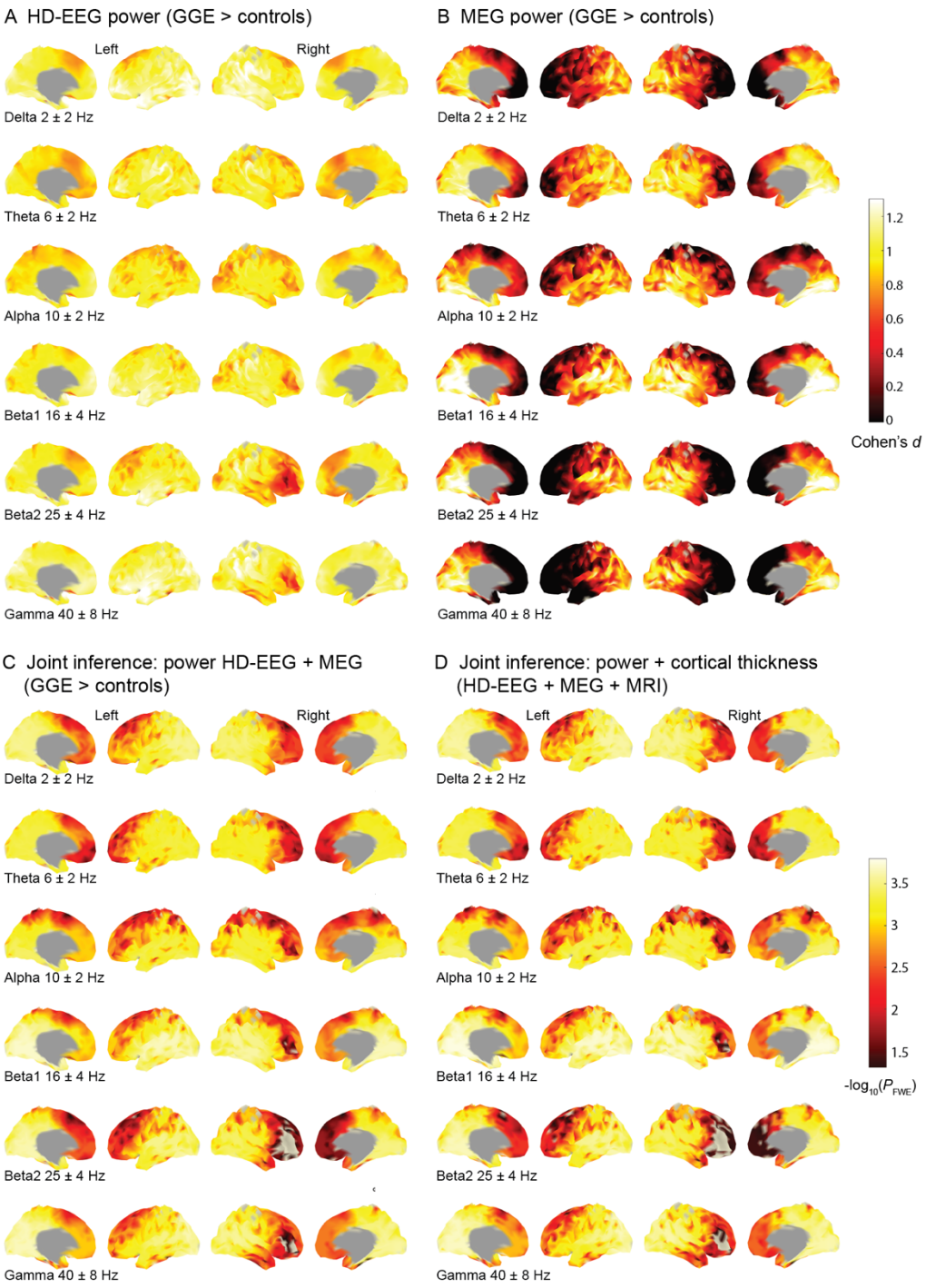

FIGURE 3 Effect sizes of separate EEG and MEG power increases in GGE, and joint inference with and without cortical thickness

(A) and (B) show standardized effect sizes (Cohen's *d*) for increased power in patients with GGE (n = 23) versus controls (n = 35) using EEG and MEG, respectively. Effect sizes were derived from the *t*-values of the permutation analyses of linear models. *d* is therefore adjusted for age, sex, scanner, and intracranial volume effects. $d = 0.2$ indicates a small effect, $d = 0.5$ a medium, and $d \geq 0.8$ a large effect. [27] The power increases in GGE patients were significant across the frequency spectrum with a

stronger posterior focus in MEG compared with EEG. (C) The power increases in the joint analysis of EEG and MEG were prominent in the occipital and temporoparietal regions. (D) The inclusion of cortical thickness in the joint analysis of EEG and MEG patterns resulted in more pronounced power differences between patients and controls in temporoparietal regions. We used a non-parametric combination of EEG/MEG connectivity and cortical thickness based on Fisher's method[25] with age, sex, scanner, and intracranial volume as covariates of no interest.

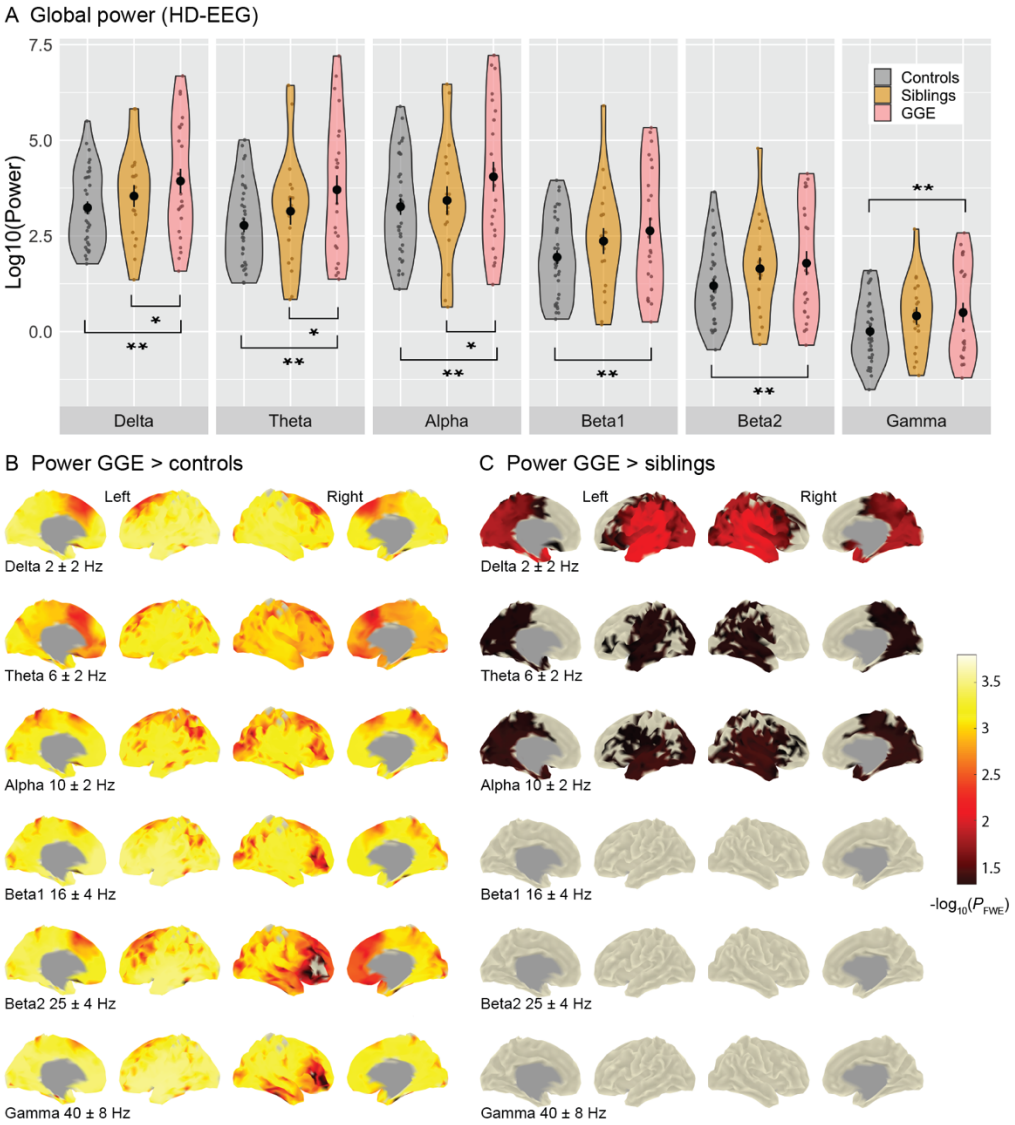

FIGURE 4 EEG analyses of global and vertex power

(A) Individual global power values of controls (n = 35), siblings (n = 18), and patients with GGE (n = 23). Shown are the density of data, group means, and standard errors of means. Statistically significant group differences are marked with an asterisk (*p < 0.05, **p < 0.001). See Table S2 for more detailed results. (B) and (C) Significantly increased vertex power is highlighted in patients compared with controls, and in patients compared with siblings, respectively, at a -log10 p threshold of 1.3 (equivalent to p < 0.05 family-wise error corrected). We used a permutation analysis of linear models for global and vertex analyses with age, sex, scanner, and intracranial volume as covariates of no interest.

Joint inference of functional and structural metrics

The inclusion of cortical thickness in the joint connectivity analysis increased the statistical significance of group differences between patients and controls. This was particularly the case for the theta band in frontocentral and temporal regions (Figure 1D), with an average decrease of significant *p*-values by 3 -$\log_{10}$-levels (Figure 5A). *P*-values were also lower for the delta and beta1 contrast as well as in the alpha band (differences of 2-2.5 -$\log_{10}$-levels). In higher frequency bands, the group contrasts became mainly stronger in superior frontal regions (beta2, gamma; difference of ~1.5 -$\log_{10}$-levels).

Power contrasts became stronger in all frequency bands and in occipital-parietal and central areas of the brain (Figure 3D and Figure 5A; differences of ~4 -$\log_{10}$-levels). Group comparisons between siblings and controls or patients and siblings did not change significantly when cortical thickness was taken into account.

Cortical thickness in GGE and siblings

Separate analyses in patients, siblings, and controls did not reveal significant differences between the groups (at vertex-level and globally, $p > 0.05$). Regional standardized group mean differences, however, suggest cortical thinning in patients predominantly in the right hemisphere and paracentral and precentral gyri ($d > 0.5$), and in frontoparietal regions and cuneus ($d > 0.4$; Figure 5B). These patterns were significant only in the uncorrected maps ($p_{uncorr} < 0.05$). Siblings had lower cortical thickness than controls in frontocentral regions ($d > 0.3$; Figure 5B). In the right supra marginal gyrus, right temporal gyri and left medial orbitofrontal regions the siblings had a greater cortical thickness than controls ($d > 0.4$), but the patterns did not survive corrections for multiple comparisons and were not present in the patients.

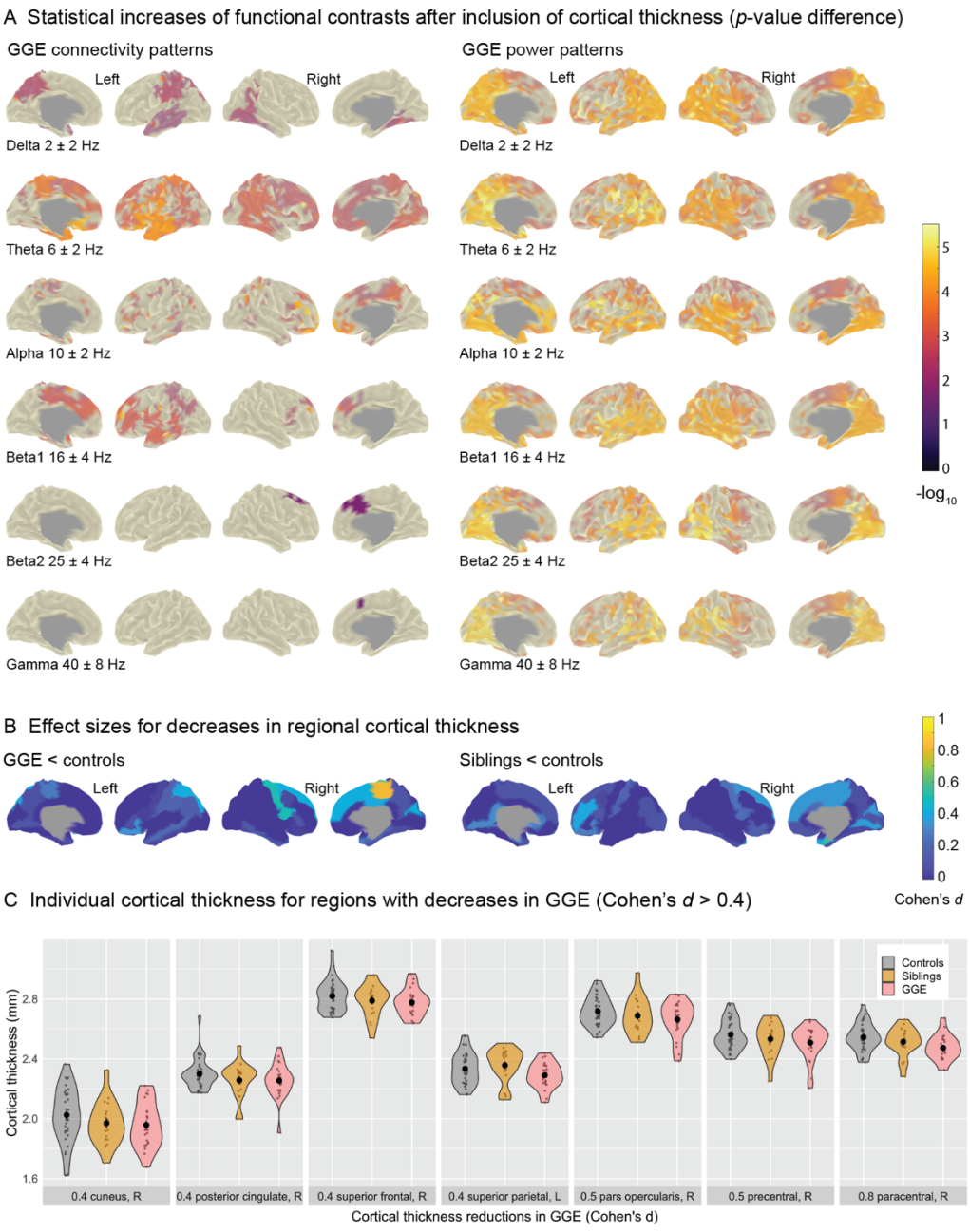

FIGURE 5 Effects of cortical thickness on joint inference analysis and group-level differences

(A) When cortical thickness was jointly analyzed with functional data (EEG + MEG), the connectivity patterns of GGE patients were amplified mainly in the theta and beta frequency band. The power patterns became stronger in temporoparietal and occipital regions. The plot shows significant vertices with an increase in the *p*-value (-log10 difference) after adding cortical thickness to the joint functional analysis. (B) The plot shows standardized effect sizes (Cohen's *d*) for reduced cortical thickness in patients with GGE (n = 23) against controls (n = 35), and in siblings (n = 18) against controls, respectively. Effect sizes were derived from the *t*-values of the permutation analysis of linear models in cortical regions (Desikan-Kiliany atlas)[28]. *d* is therefore adjusted for

age, sex, scanner, and intracranial volume effects. $d = 0.2$ indicates a small effect, $d = 0.5$ a medium and $d \geq 0.8$ a large effect.[27] (C) The violin plot shows individual thickness values for cortical regions, in which patients with GGE had lower cortical thickness than controls (Cohen's $d > 0.4$). Individual cortical thickness values were adjusted for effects of age, sex, scanner, and total intracranial volume.

Clinical factors

To assess whether the intake of medication influenced the structural and functional metrics, we split the patient group according to AED exposure. Functional connectivity was generally lower in patients taking two or more drugs (n = 6) than in patients taking none or one drug (n = 17). This finding was observed using both, EEG and MEG, and was significant in the beta1 band of vertex-level analyses (separate and joint inference, Figure 6B) and global analyses (global EEG: t_{17} = -1.94, p = 0.039, d = - 0.94; MEG: t_{17} = -1.83, p = 0.046, d = - 0.89; joint inference p = 0.015). The global EEG connectivity mean of the patients with high drug exposure was also lower than the mean of the controls and siblings (mainly in the delta, beta1, beta2, and gamma bands, Figure 6A), which was not the case in the MEG analysis (not shown). Neither EEG nor MEG power of the patients differed significantly with respect to drug exposure (vertex-level and globally, $p > 0.05$). Cortical thickness did not differ in relation to AED exposure (vertex-level and globally, $p > 0.05$).

The assessment of other clinical variables, such as the occurrence of GSWD during EEG/MEG recordings or seizure control, was complicated by the small sample sizes, unequal sex ratios and age distributions in the corresponding patient subgroups. Because EEG power differed between sexes and cortical thickness varied with age, we were unable to distinguish these effects from the effects of interest.

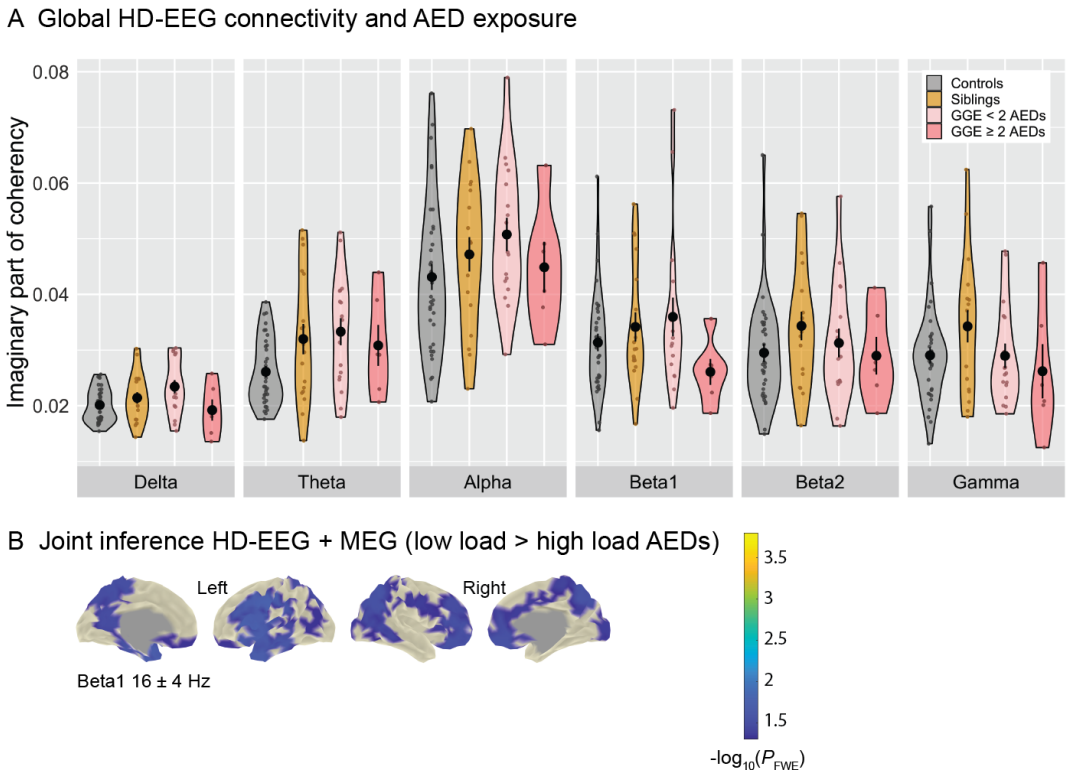

FIGURE 6 Antiepileptic medication and decreased connectivity in GGE

(A) Individual global connectivity for separate EEG analyses in controls (n = 35), siblings (n = 18), and patients with low (n = 17) and high drug load (n = 6). The violin plots show the density of data, group means, and standard errors of means. Global and (B) vertex connectivity in the beta1 band was significantly lower in patients taking two or more antiepileptic drugs (high load) at the time of the measurement than in patients taking fewer than two drugs (low load). This effect was observed in separate EEG and MEG analyses as well as in the joint analysis, but was more pronounced in EEG than in MEG. Vertices are color-coded at a -log10 p threshold of 1.3 (equivalent to $p < 0.05$ family-wise error corrected). Results for global and vertex analyses were obtained by permutation testing of linear models with age, sex, scanner, and intracranial volume as covariates of no interest.

Discussion

We combined electrophysiological signals at rest and cortical morphology to advance our understanding of network pathology in GGE and its genetic basis. EEG detected functional alterations in GGE similar to MEG, but with a different spectral sensitivity profile. The statistical integration of both modalities suggests a significant role of theta and beta oscillations in GGE. Cortical thinning was observed in our GGE cohort and amplified the functional group contrast when jointly analyzed. Similar functional and structural characteristics in healthy siblings of the patients suggest genetic contribution to the imaging patterns.

Aberrant neuronal excitability and synchronization during the ictal state typically play a crucial role in epilepsy.[29] Even in the interictal state, patients with GGE have higher global and large-scale synchronization and power, as similarly observed using EEG and MEG but with spectral differences relevant for comparisons of clinical studies. The joint EEG/MEG analysis revealed significant connectivity increases in the delta to beta1 frequency bands, with a broader spectral distribution in the MEG, peaking in the beta1 band, as previously described in the same[2] and other cohorts.[30, 31] Conversely, EEG was more sensitive to connectivity increases in the lower frequencies, particularly in theta. This could have several reasons. In line with previous studies, high drug exposure likely had a normalizing effect[32] that was significant for the EEG beta1 band and less pronounced in the MEG analysis. It is also possible that the phase estimation in the higher EEG frequencies was noisier than with MEG, despite careful artifact suppression, possibly due to greater susceptibility to electromyogenic effects.[33, 34] While studies on electrophysiological connectivity in GGE are still limited, more is known about oscillatory power in this condition. Most resting-state EEG studies have associated GGE to power increases in the theta frequency band, followed by increases in the beta band, and mixed results for delta and alpha frequencies.[35] However, the choice of channel density, analysis space, and patient characteristics has been inconsistent across studies and presents a challenge to the comparability of results.[35] We used EEG and MEG systems with comparable channel coverage and analysis pipelines in the same individuals and observed increased power in both modalities and across the conventional frequency spectrum.

GGE phenotypes within each modality were spatially similar across the frequency spectrum. This is consistent with the idea of a timescale-invariant spatial organization of the electrophysiological connectome,[36] that is, neuronal signals likely operate within the same networks at different timescales. It is conceivable that GGE-typical changes are reflected in the broadband and similar brain regions, with a dominant role of theta and beta oscillations particularly evident in our joint EEG/MEG analysis. At this point, it should be noted that we only assessed static network function. Dynamic approaches in healthy subjects suggest transient reconfigurations of spontaneous activity in specific frequencies and networks.[37] Further, the spatial representation of GGE patterns differed between the modalities. Frontotemporal connectivity alterations were more readily detected by MEG, whereas increased EEG connectivity was more pronounced in central regions. The power patterns were more

widespread in the EEG analysis and focused on the posterior regions in the MEG analysis. This difference in focality may be due to distinct signal origins resulting in a clearer separation of cortical sources in MEG, as well as greater spatial smearing of electrical signals due to different tissue conductances.[3] Also, EEG tends to measure differently oriented sources and MEG captures mainly tangential currents originating in cortical fissures.[38] On the other hand, the signal-to-noise ratio varies depending on the modality, brain region, and frequency, which limits the comparability of the contrast maps. Also, we recorded EEG and MEG separately, and the choice of the head model and slightly different measurement conditions may have introduced a bias. Direct comparisons of simultaneously recorded M/EEG have shown that both methods measure the same intrinsic network,[39] especially between 8 and 32 Hz.[40] However, different spatial sensitivity for frontoparietal connections and effects of head model complexity were found.[39] To take advantage of both methods, we adopted a combined approach and weighted the EEG- and MEG-derived statistics equally, which provided new insights into the network characteristics of GGE.

Moreover, we provide evidence for the heritability of EEG phenotypes. The present analysis suggests an intermediate position of network levels in siblings, except for beta and gamma connectivity. Here, the global mean in the siblings was higher than that in the patients, which was probably related to medication effects in the patients. For MEG phenotypes, we previously demonstrated that heritability was strongest in beta frequencies.[2] Altogether, we have extended the results of previous endophenotype research in GGE and JME using (f)MRI[11-15] by showing that resting-state alterations measured at high temporal resolution co-segregate in families with affected members. A recent genetic correlation study supports the notion of an endophenotype based on fast brain oscillations. Genetic risk for increased theta and beta power, as measured at the vertex (Cz) electrode, was associated with a higher risk for GGE.[41] Beta oscillations have been linked to motor control[42] and GABAergic mechanisms,[43] which in turn play a meaningful role in the pathology of GGE.[44] Theta rhythms are thought to be involved in hippocampal networks.[45] In animal models of temporal lobe epilepsy, increased theta synchronization in the transition phase to seizures[46] and coupling to the prefrontal cortex have been observed.[47]

When cortical thickness was added to the connectivity analyses, the differences between patients and controls were amplified, especially in the theta and beta bands, and anterior and central brain regions. Power contrasts in posterior regions were also strengthened, suggesting a relationship between aberrant morphology and functional organization in GGE. Clearly, this interplay requires further investigations. The spatial correspondence between fMRI connectivity and cortical atrophy in GGE has been studied earlier, but without significant results.[8] We did not directly assess the structure-function relationship, but statistically combined multiple modalities, which can provide greater power than separate analyses.[24] Thus, it is easier to detect a true effect that acts on all measured characteristics simultaneously.[24] Indeed, a separate analysis of cortical thickness revealed a reduction in patients with a central focus consistent with earlier findings[6, 8], but significance did not survive corrections for multiple testing. Yet, the functional contrasts benefited from taking cortical thickness into account, which argues

for the integration of the three modalities to improve diagnostic and prediction accuracy.[48] In addition, cortical thinning in GGE may not simply reflect disease activity but also genetic background. In our study, siblings without epilepsy also had reduced cortical thickness in the superior frontal and paracentral areas, arguing against a change subsequent to seizures and disease progression alone. A larger sample is needed to validate these results, but strong evidence for genetic risk signals enriched in the frontal cortex has also been demonstrated in a recent genome-wide mega-analysis.[49] Other markers such as prefrontal and cingulate curvature and surface area have been proposed as endophenotypes in JME, [7] but these likely underlie other neurodevelopmental trajectories.[50]

Overall, the integration of MEG and EEG resting-state signatures and brain morphology provided valuable information concerning GGE pathophysiology. Our investigations in healthy siblings without active epilepsy suggest that the observed imaging phenotypes are likely genetically driven. These findings pave the way to advance the deciphering of the genetic predisposition to GGE using imaging metrics.

Key Points

- Interictal states in GGE were characterized by widespread increases in fast oscillatory activity and synchronization
- Network conditions were similarly detected by EEG and MEG, but with spectral and spatial differences
- GGE network changes were reflected in the broadband, but particularly in theta and beta frequencies
- Cortical thinning in GGE was related to functional patterns and amplified group contrasting
- Similar structural and functional phenotypes in healthy siblings suggest a genetic influence

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Conflict of Interest

N.K.F. has received honoraria from Arvelle, Bial, Eisai and EGI-Phillips, all unrelated to the current work. J.M. received lecture fees and travel support by UCB, Eisai, Desitin, Alexion, GW-Pharma and the German society for ultrasound (DEGUM), all unrelated to the current study. H. L. received speaker bureau and consultancy fees from Arvelle, Bial, Biomarin, Eisai and UCB, all unrelated to this study. C.S., M.L., R.K., A.E., Y.L., and C.B. report no disclosures. We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

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Supplementary Materials for:

Title: Combined electrophysiological and morphological phenotypes in patients with genetic generalized epilepsy and their healthy siblings

Authors: Christina Stier[1, 2], Markus Loose[1], Raviteja Kotikalapudi[1, 2, 3], Adham Elshahabi[2,4], Yiwen Li Hegner[2], Justus Marquetand[2, 5], Christoph Braun[2, 6, 7], Holger Lerche[2], Niels K Focke[1, 2]

[1] Clinic of Neurology, University Medical Center Göttingen, Göttingen, Germany
[2] Department of Neurology and Epileptology, Hertie Institute for Clinical Brain Research, University of Tübingen, Tübingen, Germany
[3] Institute of Psychology, University of Bern, Bern, Switzerland
[4] Department of Neurology, University Hospital Zurich, Zurich, Switzerland
[5] Department of Neural Dynamics and Magnetoencephalography, Hertie-Institute for Clinical Brain Research, University of Tübingen, Tübingen, Germany
[6] MEG-Center, University of Tübingen, Tübingen, Germany
[7] CIMeC, Center for Mind/Brain Sciences, University of Trento, Rovereto, Italy

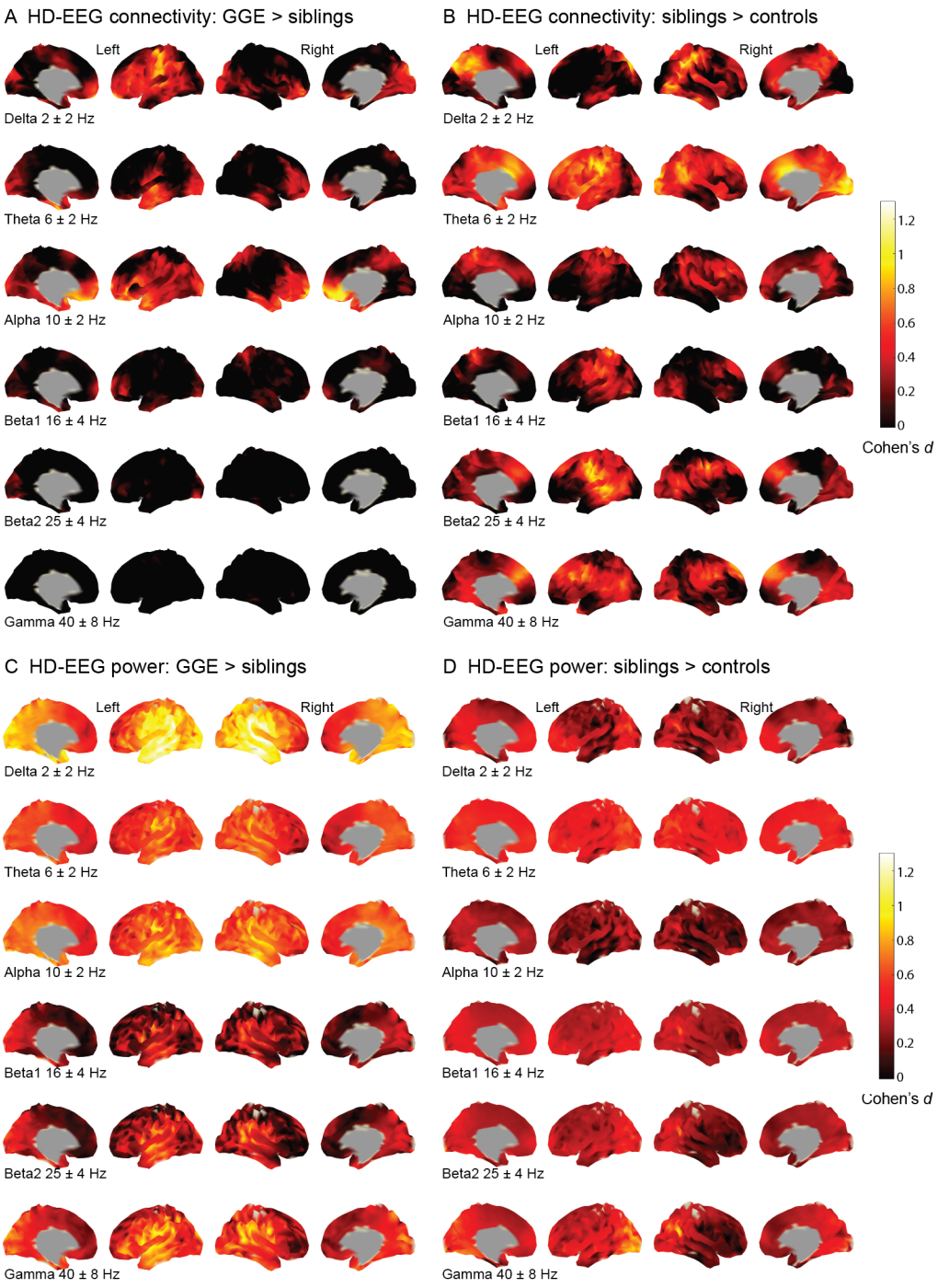

Figure S1 Effect sizes for group differences

The plot shows effect sizes (Cohen's *d*) for increased connectivity in (A) patients with GGE versus siblings, and (B) siblings versus controls as well as for increased power in (C) patients with GGE versus siblings, and (D) siblings versus controls. Effect sizes were derived from the t-values of the permutation analyses of linear models. *d* is therefore adjusted for age, sex, scanner, and intracranial volume effects. $d = 0.2$ indicates a small effect, $d = 0.5$ medium, and $d \geq 0.8$ a large effect (Cohen, 1992). Sample sizes: patients with GGE $n = 23$, siblings $n = 18$, controls $n = 35$.

TABLE S1 Clinical details of the patients with genetic generalized epilepsy.

Subject ID	Age	Sex	Syndrome	Ever had absences	Ever had GTCS	Age at onset	Disease duration	Seizure control	Drug intake at measurement (dose mg)	Previous drug intake (dose mg)	GSWD in routine/ longterm EEG	GSWD during M/EEG recordings	Family history epilepsy
1	25	F	GGE-GTCS	no	yes	16	9	yes	LEV(1000)	LEV(3000), VPA(600)	yes	no	negative
2	18	F	JME	no	yes	17	1	no	none	none	yes	no	negative
3	27	M	GGE	unclear	yes	17	10	no	VPA(1200)	VPA(750)	yes	yes	positive
4	35	M	JAE	yes	yes	15	20	yes	VPA(2000), LEV(1000)	none	yes	no	negative
5	36	F	JAE	yes	yes	16	20	yes	VPA(1000)	none	yes	yes	negative
6	21	M	JME	no	yes	17	4	yes	VPA(1000)	none	yes	no	negative
7	49	F	JAE	yes	yes	14	35	yes	VPA(900)	none	yes	no	negative
8	19	M	JAE	yes	no	17	2	no	none	none	no	no	positive
9	50	F	JME	no	yes	24	26	yes	TPM(200)	PHT(100), PB(750)	yes	no	negative
10	25	F	JME	no	yes	17	8	yes	LEV(1000)	none	no	no	positive
11	25	F	CAE	yes	yes	6	19	yes	VPA(900), ESM(750)	LTG(400), LEV(3000)	yes	no	positive

Subject ID	Age	Sex	Syndrome	Ever had absences	Ever had GTCS	Age at onset	Disease duration	Seizure control	Drug intake at measurement (dose mg)	Previous drug intake (dose mg)	GSWD in routine/ longterm EEG	GSWD during M/EEG recordings	Family history epilepsy
12	22	M	GGE	no	yes	15	7	no	LEV(3000)	LTG(400)	yes	yes	positive
13	42	M	JME	no	yes	18	24	yes	LEV(3000), VPA(600)	LEV(3000), LTG(200)	yes	no	positive
14	49	F	GGE	no	yes	10	39	yes	LTG(300)	PRM(unk.)	no	no	positive
15	19	F	GGE-GTCS	no	yes	14	5	yes	LTG(500)	none	yes	no	positive
16	37	M	JAE	yes	yes	10	27	no	LTG(600)	VPA(1200), ESM(unk.), PB(unk.), LEV(2000)	yes	no	positive
17	26	F	CAE	yes	yes	9	17	yes	LTG(600), ESM(500)	LTG(600)	yes	yes	positive
18	20	F	CAE	yes	yes	8	12	no	LTG(250), ZNS(400), LCM(300)	VPA(1050), LTG(400), LEV(2000), ESM(500)	yes	yes	negative
19	43	F	GGE	yes	yes	12	31	yes	ESL(800), LEV(1000)	LCM(unk.), CBZ(unk.), VPA(unk.)	yes	yes	positive
20	23	F	GGE-GTCS	no	yes	15	8	no	LTG(250)	none	yes	yes	negative

21	47	M	GGE-GTCS	no	yes	38	9	no	LEV(2000)	LTG(350), CLB(unk.)	yes	no	negative
22	32	F	GGE	yes	yes	8	24	yes	LEV(1000)	LTG(200)	yes	no	negative
23	24	F	CAE	yes	yes	6	18	yes	ESM(unk.)	ESM(unk.)	yes	yes	positive

CAE childhood absence epilepsy, GTCS generalized tonic clonic seizures only, GGE generalized epilepsy unclassified, JAE juvenile absence epilepsy, JME juvenile myoclonic epilepsy.

CBZ Carbamazepine, CLB Clobazam, ESM Ethosuximide, ESL Eslicarbazepine, LCM Lacosamide, LEV Levetiracetam, PB Phenobarbital, PHT Phenytoin, PRM Primidon, LTG Lamotrigine, TPM Topiramat, VPA Valproate, ZNS Zonisamide.

Unk. unknown

GSWD generalized spike-wave discharge

TABLE S2 Group-level statistics of global connectivity and power

	GGE > Controls					GGE > Siblings					Siblings > Controls				
	EEG		MEG		Joint inference	EEG		MEG		Joint inference	EEG		MEG		Joint inference
	d	p_{FWE}	*d*	p_{FWE}	p_{FWE}	*d*	p_{FWE}	*d*	p_{FWE}	p_{FWE}	*d*	p_{FWE}	*d*	p_{FWE}	p_{FWE}
Connectivity															
Delta	0.61	0.018	0.65	0.004	0.002	0.20	0.316	0.49	0.037	0.083	0.33	0.095	-0.05	0.212	0.093
Theta	0.95	0.0001	0.71	0.006	0.001	0.01	0.352	0.25	0.148	0.244	0.67	0.059	0.05	0.282	0.075
Alpha	0.62	0.017	0.59	0.023	0.009	0.26	0.302	0.53	0.038	0.118	0.25	0.156	-0.04	0.358	0.244
Beta1	0.14	0.324	1.00	<0.0001	0.003	-0.07	0.480	0.14	0.226	0.334	0.12	0.574	0.35	0.108	0.302
Beta2	0.15	0.336	0.67	0.016	0.034	-0.37	0.832	0.67	0.042	0.100	0.32	0.322	0.06	0.282	0.332
Gamma	-0.07	0.628	0.72	0.010	0.046	-0.65	0.976	0.52	0.083	0.252	0.39	0.184	0.06	0.608	0.358
Power															
Delta	1.11	<0.0001	0.85	0.002	<0.001	0.83	0.009	0.57	0.071	0.009	0.39	0.164	-0.02	0.282	0.196
Theta	0.94	<0.0001	1.12	0.001	<0.001	0.58	0.043	0.42	0.124	0.063	0.52	0.118	0.30	0.150	0.130
Alpha	0.99	0.0001	1.04	0.001	<0.001	0.71	0.022	0.72	0.020	0.013	0.31	0.22	-0.03	0.436	0.302
Beta1	1.09	<0.0001	1.33	<0.0001	<0.001	0.24	0.164	0.33	0.024	0.050	0.37	0.206	0.52	0.051	0.060
Beta2	1.06	<0.0001	1.03	0.001	<0.001	0.28	0.154	0.23	0.080	0.077	0.32	0.306	0.58	0.058	0.083
Gamma	1.17	<0.0001	0.73	0.011	<0.001	0.53	0.066	0.39	0.086	0.035	0.39	0.194	0.26	0.160	0.140

Note. Results were obtained by permutation-based testing of linear models for separate EEG/MEG analyses or by a nonparametric combination of both modalities based on Fisher's method[24] (joint inference in PALM)[23]. In all analyses, age, sex, scanner and total intracranial volume were included as covariates of no interest. According to Cohen,[26] $d = 0.2$ indicates a small effect size, $d = 0.5$ a medium, and $d \geq 0.8$ a large effect. *d* = Cohen's *d* (adjusted for covariates); FWE = family-wise error correction.

4.3 LIFESPAN TRAJECTORY OF OSCILLATORY POWER AND PHASE

Lifespan trajectory of oscillatory power and phase

Christina Stier[1, 2], Christoph Braun[2, 3, 4], Niels K Focke[1, 2]

[1] *Clinic of Neurology, University Medical Center Göttingen, Göttingen, Germany*
[2] *Department of Neurology and Epileptology, Hertie Institute for Clinical Brain Research, University of Tübingen, Tübingen, Germany*
[3] *MEG-Center, University of Tübingen, Tübingen, Germany*
[4] *CIMeC, Center for Mind/Brain Sciences, University of Trento, Rovereto, Italy*

Abstract

Objective: Development and aging are critical processes for the maturation of behavioral, socio-emotional skills and cognition, but also for the emergence of many neurological and mental diseases. While there is some evidence of structural and functional changes in the brain over the life course that were quantified primarily by (functional) magnetic resonance imaging (f)MRI, little is known about how rapid brain oscillations alter from adulthood into old age. Oscillatory power and phase synchronization map neuronal dynamics at different temporal scales and are widely studied for the description of the healthy or diseased brain.

Methods: To study the effects of age on power and phase-based connectivity, we examined data from the large-scale cross-sectional project of the Cambridge Centre for Aging and Neuroscience (Cam-CAN). For our analyses, we studied a sex-balanced group of 350 individuals aged 18 to 88 years using magnetoencephalography (MEG) during resting-state and with eyes-closed. The imaging markers were investigated in six frequency bands (~2-40 Hz) and tested for linear and quadratic association with age. Further, we estimated interaction effects of sex and age on power and connectivity.

Results Delineated age-related patterns were observed for both imaging metrics. Significant linear age effects on connectivity showed either a positive association in theta and gamma frequencies or a negative association in alpha and beta, respectively, for posterior brain areas. In the beta bands, an inverted U-shaped model was significant for connectivity in frontotemporal and central regions. Significant linear age effects on power showed a decrease in the delta band in the cingulate and a gradual increase with higher frequencies emphasized in insular and central regions. The quadratic models for power were significant for a U-shaped course in frontal delta and an inverted U-shaped relationship in higher beta and gamma encompassing cingulate and central areas. The lifespan trajectory differed between the sexes for delta power and connectivity, and theta connectivity.

Significance: We provide insights into linear and nonlinear trajectories of electrophysiological signals from adulthood into old age and show frequency-specific patterns for lower- and higher-order brain areas. Our results agree well with previous findings from fMRI but reveal temporally and spatially fine-grained patterns, encouraging further investigations of the role of electrophysiological markers at different frequencies in developmental and aging processes. The associations between oscillatory features and age diverged between males and females in the lower frequency ranges, potentially due to developmental changes in early or old adulthood.

Introduction

Brain development and aging are subject to highly complex processes that are shaped by genetic and environmental influences and are critical factors in health or disease. During development cell growth, synaptogenesis, and myelination are among the fundamental processes (Huttenlocher, 1979; Miller et al., 2012; Whitaker et al., 2016), whereas synaptic pruning, inflammatory processes, and changes in metabolism are associated with aging (Higgins-Chen et al., 2021; Huttenlocher, 1979; Silk & Wood, 2011). Early brain maturation has been related to a gradual consolidation of brain networks with association hubs becoming more strongly connected until adulthood (Oldham et al., 2022). With old age, functional connectivity within brain networks as studied using fMRI appears to decrease, particularly in the default mode network (DMN), including higher-order brain areas, and relate to cognitive decline (Damoiseaux, 2017; Dennis & Thompson, 2014; Ferreira & Busatto, 2013). This finding is thought to be in line with the hypothesis of a longer maturation period for association cortices than for primary cortices (Ferreira & Busatto, 2013; Grieve et al., 2005; Kalpouzos et al., 2009; Terribilli et al., 2011) and the particular vulnerability of these cortices to developmental disruption (Sydnor et al., 2021). Other fMRI networks such as attention, salience, or motor networks also appear to be affected during the life course, but the evidence is inconclusive (Damoiseaux, 2017; Huang et al., 2015; Onoda et al., 2012; Tomasi & Volkow, 2012).

To date, functional brain alterations across the entire lifespan have been studied primarily with fMRI. However, cerebrovascular function changes with age and can substantially confound fMRI connectivity measures if not corrected appropriately (Tsvetanov et al., 2015). MEG, on the other hand, is less sensitive to vascular confounds (Tsvetanov et al., 2015), measures neuronal activity more directly than fMRI and can capture fast neuronal dynamics. Hence, it is of great interest to track the brain's functional profile across the lifespan using electrophysiology, which has been extensively used in clinical and cognitive neuroscience to study oscillatory activity and synchronization (Sadaghiani et al., 2022; Uhlhaas & Singer, 2006). Neuronal synchronization has been related to information processing and communication in the brain (Başar & Güntekin, 2012; Buzsaki, 2006; Fries, 2005, 2015; Jokisch & Jensen, 2007), and disruptions have been linked to a range of cognitive disorders and clinical diseases (Uhlhaas & Singer, 2006). Moreover, brain oscillations at rest are genetically influenced (Glahn et al., 2010; Smit et al., 2005; Tang et al., 2007; Van Beijsterveldt et al., 1996; Zietsch et al., 2007) and likely vary with changing complex gene expression throughout life (Colantuoni et al., 2011). The onset of various disorders with significant genetic components often coincides with specific age windows, indicating alterations of developmental or aging pathways and genetic programs. For example, aberrant MEG power and synchronization have been reported for genetic generalized epilepsy, an epilepsy syndrome with typical age at onset during child- or adulthood (Elshahabi et al., 2015; Hegner et al., 2018; Stier et al., 2021; Vorderwülbecke et al., 2021). Resting-state functional MEG alterations have also been found in psychiatric disorders that occur at younger ages, such as schizophrenia (Grent et al., 2016; Hirvonen et al., 2017), as well as in diseases of aging, such as Alzheimer's disease (Kurimoto et al., 2008; Stam et al., 2002). Thus, understanding "normative" brain maturation and aging based on neuronal activity and synchronization is key to estimating pathological disease trajectories and of great importance for an increasingly aging global population (World Health Organization, 2020).

Previous electrophysiology studies have quantified oscillatory power or connectivity only for specific age ranges (Coquelet et al., 2020; Hunt et al., 2019; Marek et al., 2018; Schäfer et al., 2014) or over the lifespan, but then only at the scalp level, without providing source information on the alterations (Gómez et al., 2013; Sahoo et al., 2020). Here, we looked into the distribution of MEG power and phase-based connectivity in the population-based Cam-CAN cohort with individuals aged 18 to 88 years. Our goal was to investigate regional alterations in characteristics of brain oscillations during the resting-state using surface-based source reconstruction methods, resolved for six conventional frequency bands. Based on earlier reports of linear and non-linear findings for age effects on functional brain features (Gómez et al., 2013), we expected linear and quadratic relationships between age and power and connectivity, respectively. We also tested whether these trajectories differed between sexes, as males and females show sexually divergent biological variations, for instance in brain volume (Ritchie et al., 2018; Ruigrok et al., 2014) and functional within- and between network connectivity (Satterthwaite et al., 2015). Further, cognitive performance appears to differ between males and females, including attention, verbal fluency and reasoning, motor and visuospatial tasks (De Luca et al., 2003; Klenberg et al., 2001; Satterthwaite et al., 2015), which may indicate sex differences in brain network patterns.

Materials and Methods

Data and participants

In our study, we used cross-sectional open-access data provided by the Cambridge Centre for Aging and Neuroscience (Cam-CAN) data repository (available at http://www.mrc-cbu.cam.ac.uk/datasets/camcan/). The Cam-CAN project encompassed several phases, including cognitive assessments, interviews and health and lifestyle questionnaires, and structural and functional brain examinations (Shafto et al., 2014). Approximately 650 resting-state MEG data sets and anatomical scans were available from phase two of the study (Taylor et al., 2017), a subset of which we initially analyzed ($n = 448$). After excluding data with motion artifacts, noise, sleep, or failed extraction of cortical surface for source reconstruction ($n_{excluded} = 70$), 350 cleaned MEG and MRI data were randomly selected from the remaining data sets for further analysis in a balanced design. In total, we report on seven age groups, each with 50 individuals aged 18 to 88 years, divided into ten-year increments and balanced by sex (see demographic data in **Table 1**). All included individuals were cognitively healthy (Mini Mental State Examination score < 24; Folstein et al. (1975)) and free of neurological or psychiatric conditions (e.g. dementia, epilepsy, head injury with severe sequelae, bipolar disorder, schizophrenia) and substance abuse history. Individuals with communication problems (hearing, speech, or visual impairment), limited mobility, or MRI/MEG contraindications were excluded. For details on the study protocol and datasets see Shafto et al. (2014). The study was conducted in accordance with the Declaration of Helsinki (World Medical Association, 2013) and approved by the local ethics committee, Cambridgeshire 2 Research Ethics Committee. Data collection and sharing for this project was provided by the Cam-CAN.

Table 1 Demographic data of the individual age groups

		Age (years)	Sex
Age group	*N*	Mean ± SD	Males : females
18-28 years	50	24.4 ± 3.4	25 : 25
29-38 years	50	33.3 ± 2.9	25 : 25
39-48 years	50	43.6 ± 3.0	25 : 25
49-58 years	50	53.7 ± 2.8	25 : 25
59-68 years	50	63.0 ± 2.8	25 : 25
69-78 years	50	71.8 ± 2.9	25 : 25
79-88 years	50	81.5 ± 2.7	25 : 25

MRI acquisition

Anatomical data were acquired using a 3T Siemens TIM Trio scanner with a 32-channel head coil. T1-weighted images were derived from 3D MPRAGE sequences with TR=2250ms, TE=2.99ms, TI=900ms; FA=9 deg; FOV=256x240x192mm; 1mm isotropic; GRAPPA=2; TA=4mins 32s), and T2-weighted images from 3D SPACE sequences with TR=2800ms, TE=408ms, TI=900ms; FOV=256x256x192mm; 1mm isotropic; GRAPPA=2; TA=4mins 30s).

MEG acquisition

Resting-state data were recorded using a 306-channel VectorView MEG system (Elekta Neuromag, Helsinki) with 102 magnetometers and 204 planar gradiometers (sampling at 1 kHz with a 0.03 Hz high pass filter). Individuals were assessed in a seated position in a magnetically shielded room at a single site (MRC Cognition and Brain Science Unit, University of Cambridge, UK) for 8 min and 40 s. At the same time, four coils continuously measured the head position within the MEG helmet. Additionally, electrocardiogram (ECG) and electrooculogram (horizontal and vertical) were recorded to track cardiac signals and eye-movements. Individuals were instructed to keep their eyes closed and sit still.

MRI processing and individual head models

For mapping MEG sensor level data onto individual cortical surfaces and surfaces of deep brain regions, anatomical information was derived from T1- and T2-weighted images and reconstructed using FreeSurfer 6.0.0 (https://surfer.nmr.mgh.harvard.edu/). We applied surface-based mapping (SUMA; Saad and Reynolds (2012)), which resampled the cortical surfaces to 1,002 vertices per hemisphere (ld factor = 10), based on the 'fsaverage' template mesh provided by FreeSurfer and SUMA. Additionally, six deep brain structures were reconstructed (bilateral amygdala, hippocampus, thalamus, caudate, putamen and pallidum), also based on the 'fsaverage' template atlas. Each region was converted to surfaces using Matlab (isosurface) and resampled to a number of vertices corresponding to their average volume in comparison to the SUMA reconstruction (334 vertices in total; 167 per hemisphere). These reference surfaces were spatially normalized to MNI space with DARTEL normalization (Ashburner, 2007) in SPM12 (https://www.fil.ion.ucl.ac.uk/spm/software/spm12/). In a next step, individual subject MRI images were segmented (SPM12, unified segment, Ashburner and Friston (2005)) and again processed with DARTEL using the CAT12 template (Gaser & Dahnke, 2016). The individual non-linear transformations were inverted and used to bring the fsaverage-based MNI-space subcortical vertices into the individual space. Thus, this procedure resulted in 2,338 vertex positions for each individual and point-for-point anatomical correspondence among individuals for cortical (via FreeSurfer/SUMA) and deep brain regions (via SPM/DARTEL). Next, the individual meshes were realigned to the Neuromag sensor space based on anatomical landmark coordinates provided by Cam-CAN. We used the "single shell" method implemented in Fieldtrip to compute the leadfields and individual head models for MEG source projection.

MEG processing

Preprocessed MEG data was available through the Cam-CAN repository. For each dataset Elekta Neuromag Maxfilter 2.2 was applied using temporal signal space separation (10s window, 0.98 correlation limit) to remove external interference and artefacts, line noise (50 Hz and its harmonics), and to correct for bad channels and head movements. Using the Fieldtrip toolbox (Oostenveld et al., 2011), we resampled the data to 300 Hz, high-pass filtered at 1 Hz (first order Butterworth), and segmented the data into trials of 10 s length. Trials containing artifacts were removed following an automatic approach for both MEG channel types separately (see https://www.fieldtriptoolbox.org/tutorial/automatic_artifact_rejection/ for further details). In brief, the data was bandpass filtered at 110 to 140 Hz (9th order Butterworth) for optimal detection of muscle artifacts and z-transformed for each channel and timepoint. The z-transformed values were averaged over all channels so that artifacts could be accumulated and detected in a time course representing standardized deviations from the mean of all channels. Finally, all time points that belonged to the artifact were marked using artifact padding, and data trials whose z-values were above a threshold of 14 were excluded. The remaining data were then low-pass filtered at 70 Hz (first-order Butterworth), and independent component analysis (ICA) was applied. Ocular components were automatically identified based on their similarity to EOG channel signals (high coherence and amplitude correlation). Cardiac components were identified when highly coherent with the ECG signal or based on the averaged maximum peaks timelocked to the ECG (QRS complex, see https://www.fieldtriptoolbox.org/example/use_independent_component_analysis_ica_to_remove_ecg_artifacts/). For each data set, the automatic selection of the components was manually revised. In a few cases, we manually selected the relevant ICA components because the ECG/EOG was noisy. We visually inspected all cleaned data for quality control and rated vigilance of individuals according to the criteria of the American Academy of Sleep Medicine (https://aasm.org/). From the cleaned data, which were scored to be awake, 30 trials were randomly selected for source analysis using signals from magnetometers (102 channels) only. We used beamforming (dynamic imaging of coherent sources; Gross et al. (2001)) to project sensor data to the surface points (source space) in the frequency domain. MEG power and cross-spectral densities were computed for six conventional frequency bands (delta: 2 ± 2 Hz, theta: 6 ± 2 Hz, alpha 10 ± 2 Hz, beta1 16 ± 4 Hz, beta2 25 ± 4 Hz and gamma 40 ± 8 Hz) based on fast Fourier spectral analysis using multitapers (Discrete Prolate Spheroidal Sequences tapers). Source projection was carried out using leadfields and adaptive spatial filters (regularization: lambda = 5%). The coherency coefficient was estimated between all pairs of vertices (source points, $n = 2238$) and the imaginary part was derived to account for potential field spread (Nolte et al., 2004). The absolute imaginary part of coherency was our connectivity measure of interest, reflecting phase synchrony between signals. We averaged all connections of a vertex to obtain the overall strength of a vertex, and for each individual also across all vertices to get a global connectivity and power index.

To provide an overview of connectivity and power distributions across the age groups, the frequency spectra of each individual in this study were calculated for 1-Hz bins and averaged for each age group (**Figure 1**).

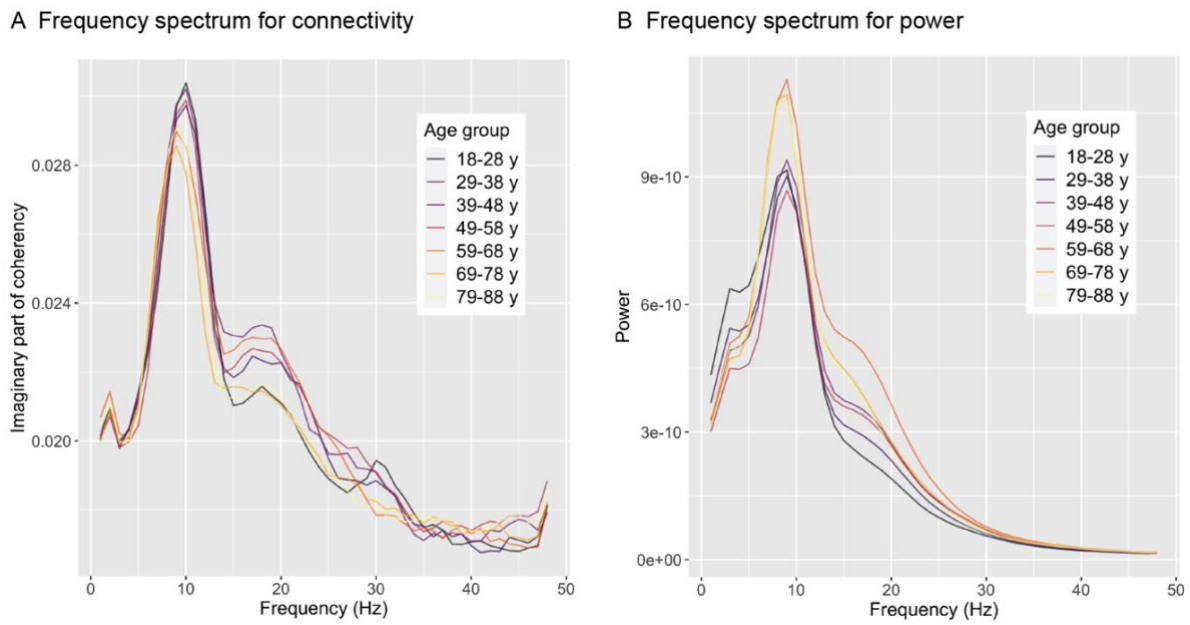

Figure 1 Spectra for global connectivity and power across age groups

For each individual included in this study, global connectivity or power was computed for 1 Hz frequency bins and averaged for the corresponding age group (see **Table 1**; n_{group} = 50). Dark colors represent the young age groups, light colors indicate the old age groups. **(A)** Mean global connectivity between the youngest and oldest age groups diverged around the alpha peak (~10 Hz). In the beta frequencies (~12-30 Hz), the mean connectivity of the youngest and the oldest was similar, whereas the middle age groups had the highest average values. In the gamma range (above ~30 Hz), the pattern is more diffuse, with the youngest age groups having lower connectivity than the older age groups. **(B)** In most frequency bands, the mean power differed across age groups. The oldest age groups had lower power than the other age groups in the delta band (~2 Hz), but higher power in all other frequency bands. Individuals under 28 years of age had the highest power in the delta band and the lowest power in the frequencies above 12 Hz compared to individuals over 28 years of age. Quantitative assessment of age-effects on power and connectivity in specific frequency bands are found in the results section, and **Figure 2** and **Figure 3**.

Statistical analysis

Linear and quadratic effects of age at the global and vertex-level

We examined the relationship between age and connectivity or power by fitting a model with the imaging metrics in each frequency band as dependent variables and age, age^2 and sex as independent variables. We centered the individual age values before squaring them to reduce the correlation between the linear and the quadratic terms. The models were estimated either for the global metrics or for the metrics at each vertex (surface point) in the brain. The non-parametric statistic tool PALM (Permutation Analysis of Linear Models, Winkler et al. (2014)) was used to generate permutations for the respective models with tail approximation for accelerated inference (500 permutations) (Winkler et al., 2016). Single *t* contrasts were computed, that is, for positive and negative linear age effects, and for convex and concave quadratic age terms. *P*-values were derived from the permutation distribution, at the tail of which a generalized Pareto distribution was fitted (Winkler et al., 2016), and corrected for multiple comparisons (family-wise error, FWE) at cluster level resulting from threshold-free cluster enhancement (Smith & Nichols, 2009). We set the significance level at $p = 0.05$ or equivalently $-\log10(p) \sim 1.3$. The partial correlation coefficient ($r_{partial}$) was estimated as an effect size for the independent variables based on the *t*-values and degrees of freedom of the global models (Rosenthal et al., 1994). $r_{partial}$ indicates the degree of association between two variables at which the influence of other variables in the model has been eliminated (Bortz & Schuster, 2011): values of ± 0.1 reflect a small effect, ± 0.3 represent a large effect, and ± 0.5 is a large effect (Field et al., 2012).

Interaction of sex and age on imaging metrics

Using PALM, we also investigated sex differences in the trajectory of imaging metrics over the lifespan. For each age decade, 25 males and 25 females were included to ensure a balanced design giving 175 individuals per sex in total. Models were fitted to test whether the beta coefficients for the age and age^2 effects on connectivity or power in each frequency band differed between males and females. Again, *p*-values were computed based on permutation analyses for the global values or vertex-values (tail approximation, 500 permutations) and family-wise error corrected at the cluster level resulting from threshold-free cluster enhancement (Smith & Nichols, 2009). We considered a threshold of $p = 0.05$ or $-\log10(p) \sim 1.3$ as significant.

Results

Global and vertex connectivity across the lifespan

Significant linear associations between age and global connectivity were observed in the theta, alpha, beta1, and gamma frequency bands **(Table 2 and Figure 2A)**. The direction for the linear association in theta and gamma connectivity was positive, and implies a global increase with age. Global connectivity in the alpha and beta1 bands decreased with age. Delta connectivity was rather stable across the age ranges ($p > 0.05$). At the vertex-level, significant linear effects were mainly observed in the occipital lobes, including cuneus and inferior parietal regions, and for the alpha band also in middle temporal regions **(Figure 2B)**. The linear age effect in the beta2 band was not significant at the global level but was significant at the vertex-level, again in occipital brain areas. Moreover, in the beta frequencies, the relationship between age and global and vertex connectivity significantly followed a quadratic function **(Table 2 and Figure 2C)**. Accordingly, middle age groups exhibited the highest global connectivity in comparison with young or old age groups. These quadratic effects, especially in beta2, were strongest in central and frontotemporal regions.

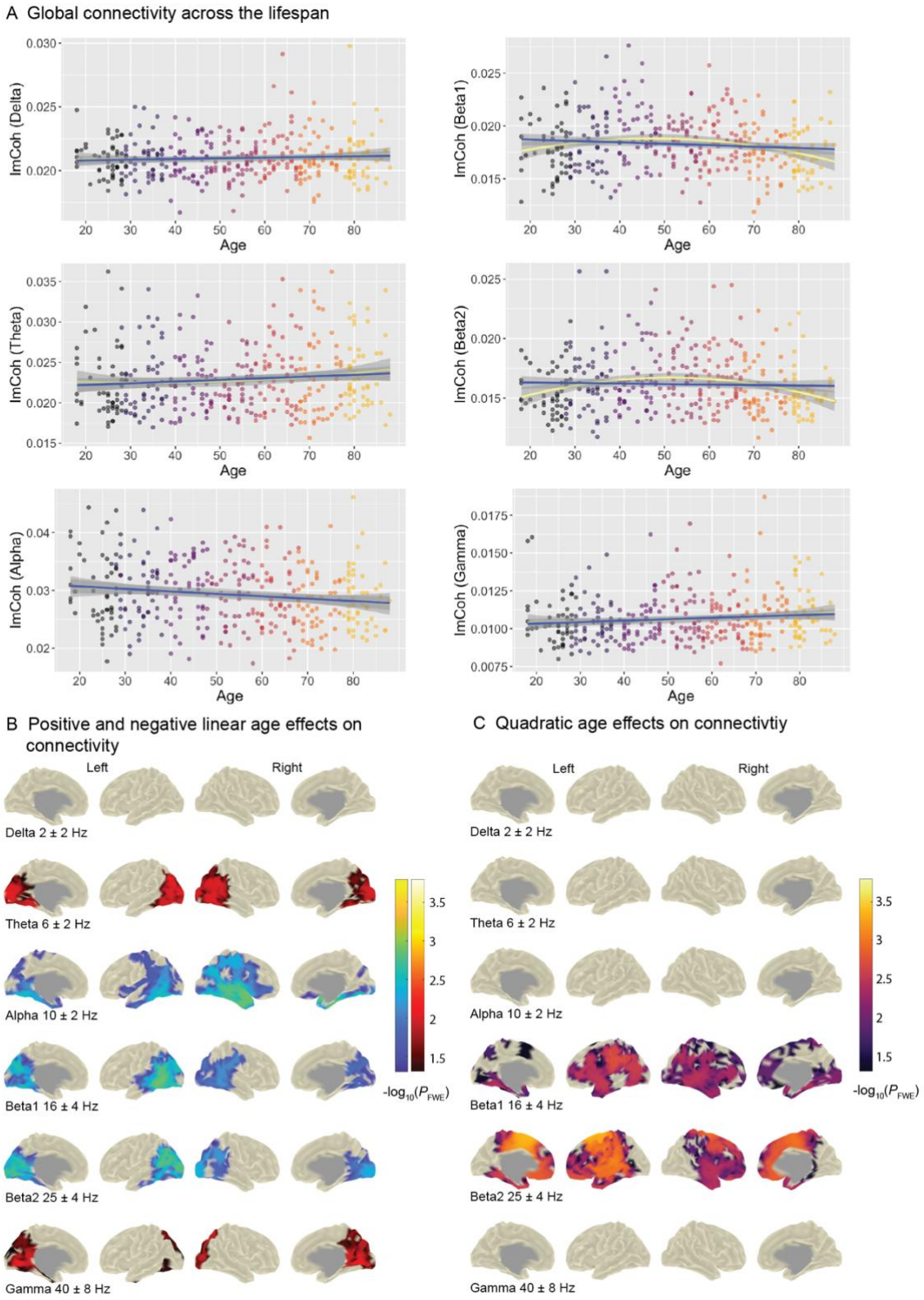

Figure 2 Frequency-specific association between age and connectivity

(**A)** The plots show individual values of global connectivity (n = 350) across early and late adulthood for the six frequency bands investigated. The blue lines represent linear relationships between age and global connectivity, while the yellow lines represent quadratic relationships. Statistical analyses yielded significant linear effects of age in the theta, alpha, beta1, and gamma frequency bands. For the beta1 and beta2 bands, the quadratic term in

the regression model was also significant. See **Table 2** for statistical details. In **(B)** and **(C)**, significant effects of age on vertex connectivity are highlighted. Vertex-results for subcortical nuclei and deep structures were not significant and are not displayed here. **(B)** the blue color bar indicates significant negative effects of age, whereas the red color bar represents significant positive associations. **(C)** The purple color bar indicates significant quadratic effects of age following an inverted U-shaped pattern (concave). Results for the U-shaped term (convex) were not significant and are not displayed. The significance level was set at -log10 $p > 1.3$ (equivalent to $p < 0.05$), family-wise error corrected. We estimated linear models for each frequency band separately with connectivity as the independent variable, and age, age2 and sex as dependent variables and performed permutation-based analysis. The included healthy individuals (n = 350) ranged in age from 18 to 88 years. ImCoh = imaginary part of coherency.

Table 2 Relationship between global connectivity and age

	Contrast	*t*	*p*	$r_{partial}$
Delta	Age positive	1.16	0.166	0.06
	Age negative	-1.16	0.892	-0.06
	Age^2 convex	0.47	0.346	0.03
	Age^2 concave	-0.47	0.706	-0.03
	Sex	-1.26	0.880	-0.07
Theta	Age positive	1.91	0.034*	0.10
	Age negative	-1.91	0.978	-0.10
	Age^2 convex	0.87	0.216	0.05
	Age^2 concave	-0.87	0.782	-0.05
	Sex	-1.83	0.974	-0.10
Alpha	Age positive	-2.82	0.992	-0.15
	Age negative	2.82	0.002*	0.15
	Age^2 convex	-0.16	0.564	-0.01
	Age^2 concave	0.16	0.464	0.01
	Sex	-1.66	0.972	-0.09
Beta1	Age positive	-2.02	0.972	-0.11
	Age negative	2.02	0.028*	0.11
	Age^2 convex	-3.33	1.000	-0.18
	Age^2 concave	3.33	0.002*	0.18
	Sex	0.48	0.350	0.03
Beta2	Age positive	-0.77	0.776	-0.04
	Age negative	0.77	0.194	0.04
	Age^2 convex	-3.88	1.000	-0.20
	Age^2 concave	3.88	<0.001*	0.20
	Sex	0.78	0.222	0.04
Gamma	Age positive	2.17	0.010*	0.12
	Age negative	-2.17	0.984	-0.12
	Age^2 convex	0.50	0.324	0.03
	Age^2 concave	-0.50	0.728	-0.03
	Sex	-2.23	0.982	-0.12

Results were obtained by permutation analyses of linear models with global connectivity (imaginary part of coherency) as independent variable and age, age^2 and sex as dependent variables. We estimated the effects for positive and negative associations between age and connectivity, as well as the convex (U-shaped) and concave (inverted U-shaped) quadratic relationship in each frequency band. $r_{partial}$ reflects the degree of association between connectivity and age at which the influence of the other effects was eliminated. Values of ± 0.1 reflect a small effect, ± 0.3 represent a large effect, and ± 0.5 is a large effect (Field et al., 2012). The included healthy individuals (n = 350) ranged in age from 18 to 88 years. * denotes statistical significance at $p < 0.05$.

Global and vertex power across the lifespan

The distribution of global power across age groups showed a trend of decrease or stability in the lower frequency bands and an increase in the faster oscillations **(Table 3 and Figure 3A)**. For delta power, there was a significant negative linear relationship with age, globally and in the cingulate and caudate **(Figure 3B).** In the theta band, the linear age effect was not significant at a global level, but at a vertex level in insular and frontotemporal regions. Global power in the higher frequencies, that is, alpha to the gamma band, showed a significant linear increase with age. At the vertex level, the linear effects in these frequency bands were widespread across the brain and most prominent in frontotemporal, insular, and central regions. In beta1 and gamma, significant linear age effects were also observed in deeper brain structures, such as thalamus, hippocampus, and putamen.

The quadratic association between delta power and age was also significant; in the global and vertex analyses with a strong focus in orbitofrontal regions, the insula, and temporal regions **(Figure 3C)**. There was also a significant quadratic effect in the theta band for global power and in insular and frontotemporal regions. The relationship between global power in the beta2 and gamma bands and age also followed a quadratic function. In these frequency bands, power was lowest in the young individuals, highest in middle age, and somewhat lower again in old age. The quadratic effect at the vertex level was highlighted in central areas and the cingulate.

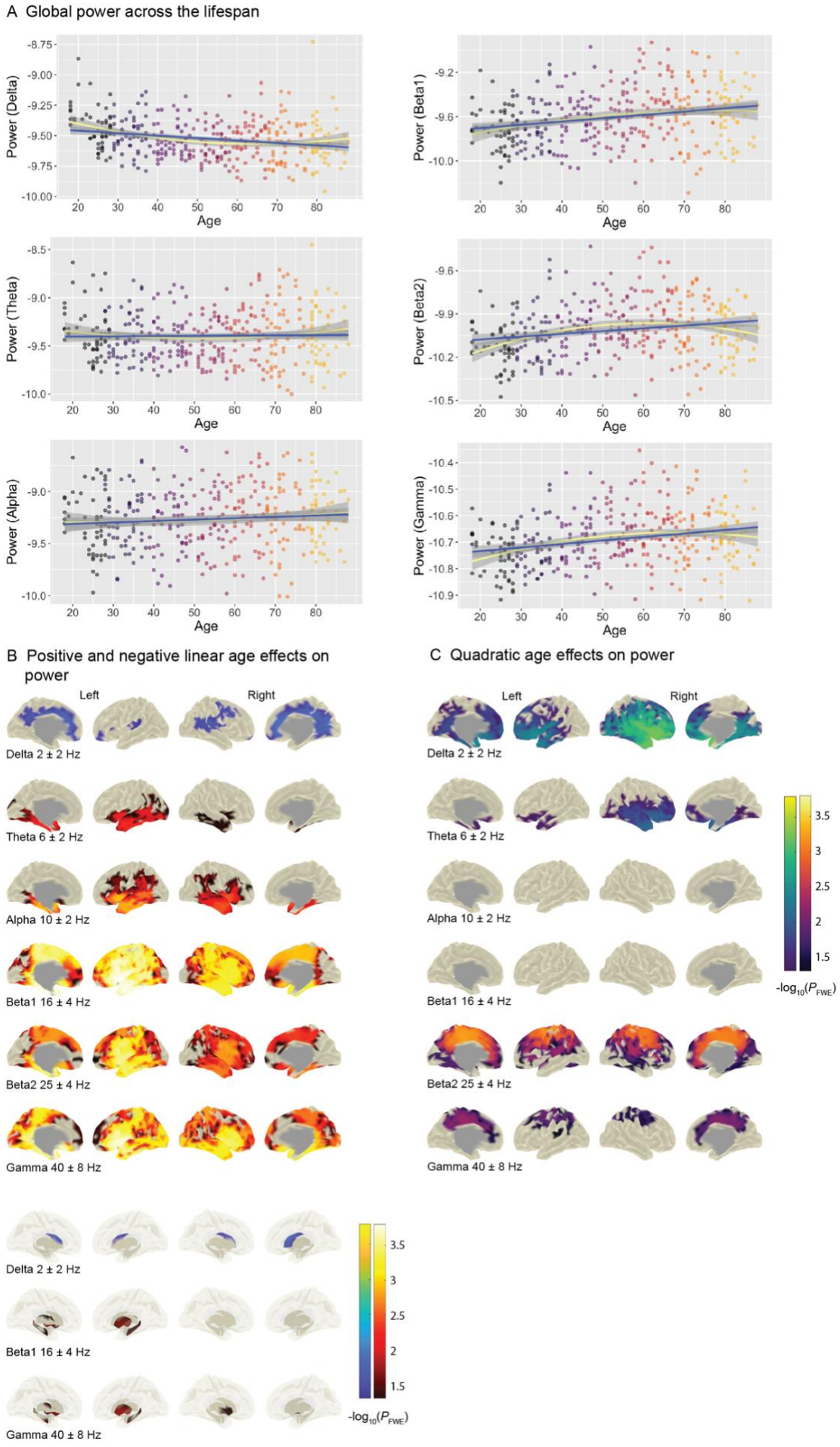
A Global power across the lifespan
Power (Delta)
Power (Beta1)
Power (Theta)
Power (Beta2)
Power (Alpha)
Power (Gamma)
Age
B Positive and negative linear age effects on power
C Quadratic age effects on power
Left
Right
Delta 2 ± 2 Hz
Theta 6 ± 2 Hz
Alpha 10 ± 2 Hz
Beta1 16 ± 4 Hz
Beta2 25 ± 4 Hz
Gamma 40 ± 8 Hz
-log10(PFWE)

Figure 3 Frequency-specific association between age and power

(**A)** The plots show individual values of global power (n = 350) across early and late adulthood for the six frequency bands investigated. The blue lines represent linear relationships between age and global power, while the yellow lines represent quadratic relationships. Power data was log10-transformed for visualization purposes. Statistical analyses yielded significant linear effects of age on global power in the delta, beta1, beta2, and gamma frequency bands. The quadratic term in the regression model was significant for the delta, theta, beta2, and gamma bands. See **Table 3** for statistical details. In **(B)** and **(C)**, significant effects of age on vertex-power are highlighted. **(B)** The blue color bar indicates significant negative effects of age, whereas the red color bar represents significant positive associations. **(C)** The green color bar depicts significant quadratic effects of age following a U-shaped pattern (convex), while the purple color bar indicates significant effects following an inverted U (concave). The significance level was set at -log10 $p > 1.3$ (equivalent to $p < 0.05$), family-wise error corrected. We estimated linear models for each frequency band separately with power as the independent variable, and age, age^2 and sex as dependent variables and performed permutation-based analysis. The included healthy individuals (n = 350) ranged in age from 18 to 88 years.

Table 3 Relationship between global power and age

	Contrast	*t*	*p*	$r_{partial}$
Delta	Age positive	-3.14	0.998	-0.17
	Age negative	3.14	<0.001*	0.17
	Age^2 convex	3.54	0.002*	0.19
	Age^2 concave	-3.54	1.000	-0.19
	Sex	-2.48	0.996	-0.13
Theta	Age positive	1.11	0.142	0.06
	Age negative	-1.11	0.874	-0.06
	Age^2 convex	2.03	0.025*	0.11
	Age^2 concave	-2.03	0.980	-0.11
	Sex	-2.20	0.990	-0.12
Alpha	Age positive	1.72	0.050	0.09
	Age negative	-1.72	0.958	-0.09
	Age^2 convex	0.10	0.482	0.01
	Age^2 concave	-0.10	0.516	-0.01
	Sex	-3.19	1.000	-0.17
Beta1	Age positive	4.44	<0.001*	0.23
	Age negative	-4.44	1.000	-0.23
	Age^2 convex	-1.57	0.938	-0.08
	Age^2 concave	1.57	0.051	0.08
	Sex	-1.45	0.936	-0.08
Beta2	Age positive	3.27	<0.001*	0.17
	Age negative	-3.27	0.998	-0.17
	Age^2 convex	-3.95	1.000	-0.21
	Age^2 concave	3.95	<0.001*	0.21
	Sex	-1.22	0.910	-0.07
Gamma	Age positive	4.66	<0.001*	0.24
	Age negative	-4.66	1.000	-0.24
	Age^2 convex	-2.76	0.996	-0.15
	Age^2 concave	2.76	0.003*	0.15
	Sex	-1.56	0.952	-0.08

Results were obtained by permutation analyses of linear models with global power as independent variable, and age, age^2 and sex as dependent variables. We estimated the effects for positive and negative associations between age and power as well as the convex (U-shaped) and concave (inverted U-shaped) quadratic relationship in each frequency band. $r_{partial}$ reflects the degree of association between power and age at which the influence of the other effects was eliminated. Values of ± 0.1 reflect a small effect, ± 0.3 represent a large effect, and ± 0.5 is a large effect (Field et al., 2012). The included healthy individuals (n = 350) ranged in age from 18 to 88 years. * denotes statistical significance at $p < 0.05$.

Sex differences for power and connectivity across the lifespan

The main effect for sex was not significant for either global connectivity or global power **(Table 2 and Table 3)**, but was significant for beta2 and gamma power at a vertex level in frontal regions (data not shown). We further tested whether the slopes for the linear and quadratic age effects differed significantly between sexes, which was the case in the lower frequency bands. For delta connectivity, there was a steeper linear decrease with age in males than in females, which was significant in occipital areas of the brain and cuneus **(Figure 4B)**, but not at a global level **(Table 4)**. In the theta band, quadratic age effects on connectivity were significantly different between the sexes at a global level, but did not survive correction for multiple comparisons in the vertex analysis. Delta power linearly decreased with age with a stronger decline for men than women. This was the case in the global analysis **(Table 4)** as well as in frontocentral regions, cingulate, and precuneus **(Figure 5B)**. In other frequency ranges for connectivity and power, there was no significant interaction between sex and age.

Table 4 Summary of interaction effects sex by age on global MEG metrics

		Connectivity		Power	
	Contrast	*t*	*p*	*t*	*p*
Delta	Sex*age positive	-1.38	0.896	-2.57	0.998
	Sex*age negative	1.38	0.081	2.57	<0.001*
	Sex*age^2 convex	-1.29	0.882	1.20	0.114
	Sex*age^2 concave	1.29	0.092	-1.20	0.872
Theta	Sex*age positive	0.95	0.186	-1.14	0.866
	Sex*age negative	-0.95	0.826	1.14	0.134
	Sex*age^2 convex	1.82	0.039*	1.55	0.052
	Sex*age^2 concave	-1.82	0.962	-1.55	0.940
Alpha	Sex*age positive	0.84	0.196	-0.16	0.564
	Sex*age negative	-0.84	0.792	0.16	0.446
	Sex*age^2 convex	-0.86	0.808	1.33	0.077
	Sex*age^2 concave	0.86	0.192	-1.33	0.930
Beta1	Sex*age positive	1.08	0.158	0.03	0.506
	Sex*age negative	-1.08	0.842	-0.03	0.500
	Sex*age^2 convex	1.29	0.102	1.39	0.081
	Sex*age^2 concave	-1.29	0.888	-1.39	0.910
Beta2	Sex*age positive	-1.10	0.870	0.08	0.486
	Sex*age negative	1.10	0.152	-0.08	0.514
	Sex*age^2 convex	0.60	0.268	1.37	0.086
	Sex*age^2 concave	-0.60	0.752	-1.37	0.912
Gamma	Sex*age positive	-0.91	0.822	0.88	0.188
	Sex*age negative	0.91	0.194	-0.88	0.806
	Sex*age^2 convex	1.59	0.071	1.63	0.056
	Sex*age^2 concave	-1.59	0.948	-1.63	0.944

Results were obtained by permutation analyses of linear models testing whether the coefficients for the age and age^2 effect on MEG metrics differed between males (*n = 175*) and females (*n = 175*). Positive and negative linear associations between age and the metrics as well as the convex (U-shaped) and concave (inverted U-shaped) quadratic relationship in each frequency band were considered. The included healthy individuals ($n = 350$) ranged in age from 18 to 88 years. * denotes statistical significance at $p < 0.05$.

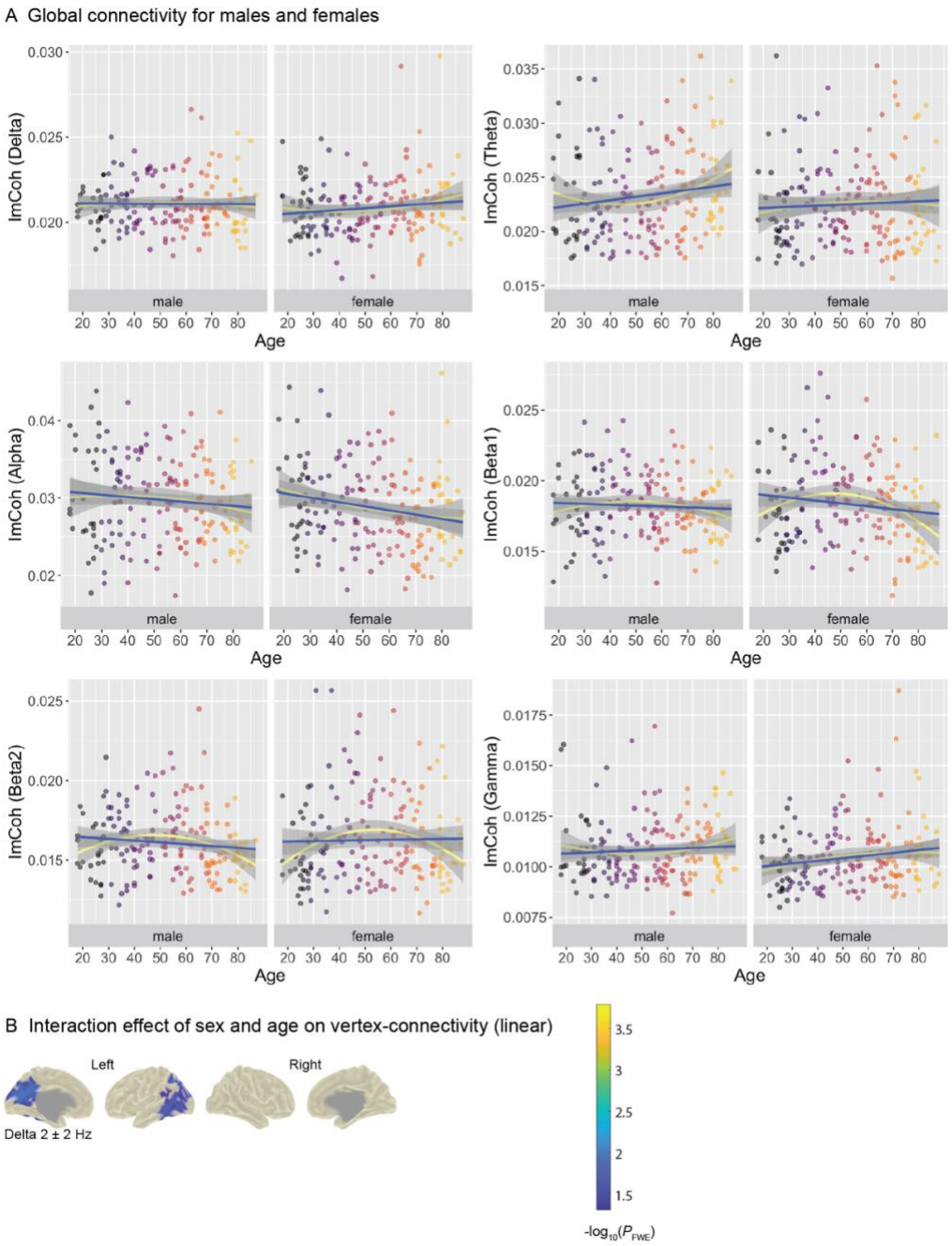

Figure 4 Sex-specific trajectories for connectivity with age

(A) The plots show individual global connectivity across early and late adulthood separately for males (n = 175) and females (n = 175) and for the six frequency bands studied. The blue lines represent linear relationships between age and global connectivity, while the yellow lines represent quadratic relationships. The interaction effect of sex and age on global connectivity was significant for the quadratic association in the theta frequency band. See **Table 4** for statistical details. **(B)** Shown is the significant interaction between sex and age for linear vertex-connectivity in the delta band. Interaction effects in the other frequency bands were not significant and are not shown. The significance level was set at -log10 p > 1.3 (equivalent to p < 0.05), family-wise error corrected. We estimated linear models to test whether the age trajectories of connectivity differed between males and females in each frequency band using permutation-based analysis. The included healthy individuals (n = 350) ranged in age from 18 to 88 years. ImCoh = imaginary part of coherency.

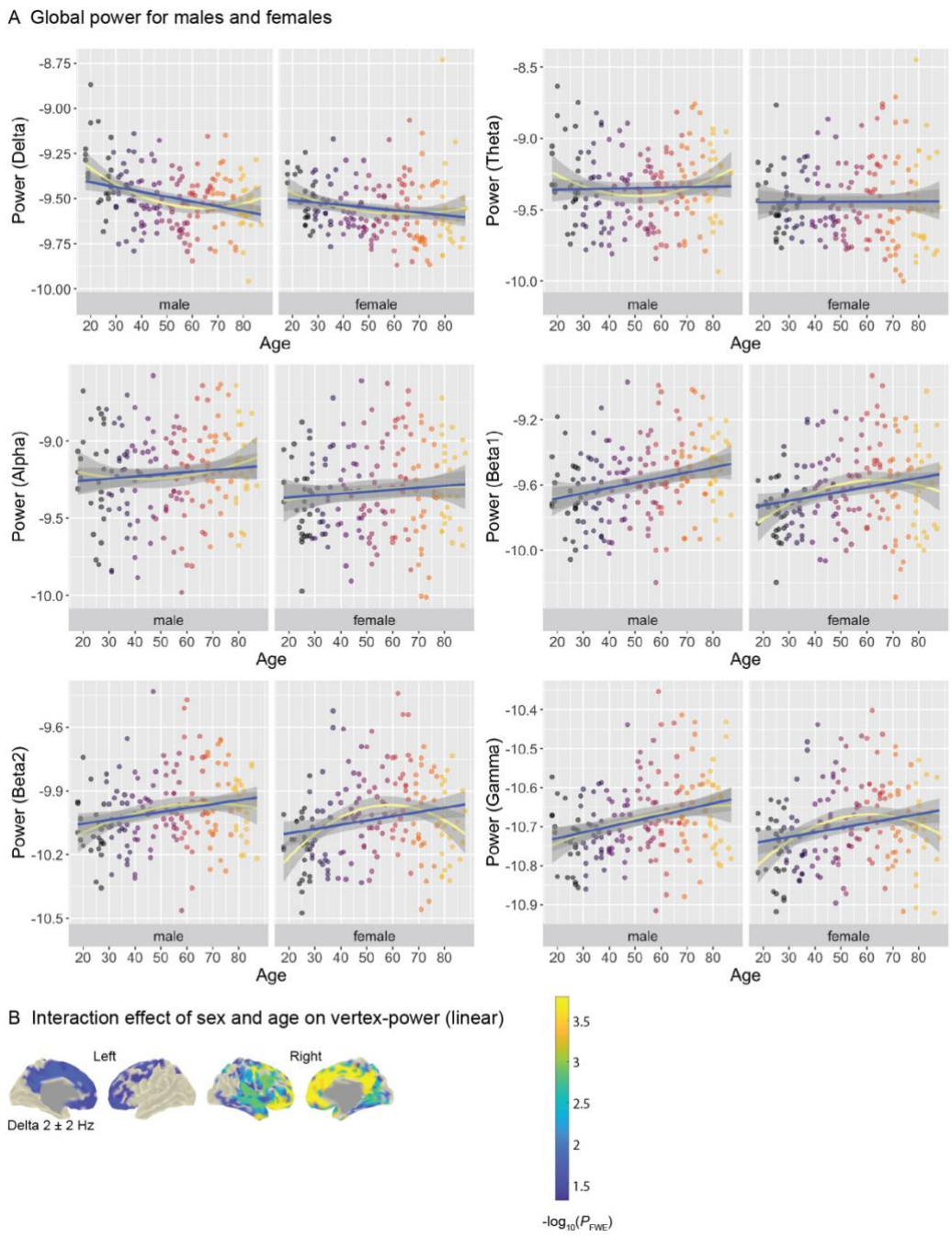

Figure 5 Sex-specific trajectories for power with age

(A) The plots show individual global power across early and late adulthood separately for males (n = 175) and females (n = 175) and for the six frequency bands studied. The blue lines represent linear relationships between age and global connectivity, while the yellow lines represent quadratic relationships. Power data was log10-transformed for visualization purposes. The interaction effect of sex and age on global power was significant for the linear association in the delta frequency band. See **Table 4** for statistical details. **(B)** Shown is the significant interaction between sex and age for linear vertex-power in the delta band. Interaction effects in the other frequency bands were not significant and are not shown. The significance level was set at -log10 p > 1.3 (equivalent to p < 0.05), family-wise error corrected. We estimated linear models to test whether the age trajectories of power differed between males and females in each frequency band using permutation-based analysis. The included healthy individuals (n = 350) ranged in age from 18 to 88 years.

Discussion

Using a large set of age-stratified MEG resting-state recordings, we gained insights into how frequency-specific phase-coupling and power is expressed in whole-brain networks from early adulthood to old age. The markers showed different associations with age, which could be described by linear and quadratic functions depending on the frequencies, and may be related to different (physiological) aging effects. The spatial patterns of these associations were largely distinct for power and connectivity, suggesting that the measures convey nonredundant information. Connectivity changed linearly with age in mainly posterior brain regions with similar topographies for the frequency bands studied, but with increases in the theta and gamma frequencies and, conversely, decreases in the alpha to beta2 frequency bands. Connectivity in central and frontotemporal regions followed a quadratic relationship with age in the beta frequencies, suggesting that these areas might undergo different maturation or aging processes than the posterior regions. The focus of age-effects on power also tended to be in the anterior and central regions. In the linear case, there were power redistributions from lower (delta decrease) to higher frequencies (theta to gamma increases) prominent in the frontotemporal and central regions, and cingulate. Quadratic age effects for delta power, and weaker also for theta power, were observed anterior-basal. In beta2 and gamma, the quadratic age effects were strongest in the cingulate und central areas, overlapping spatially, at least in part, with the quadratic age effects on beta connectivity.

In general, very few studies have investigated phase-based neuronal organization across the lifespan. One study has reported linear changes of coherence and metastability of signals in the full MEG Cam-CAN dataset at the scalp (Sahoo et al., 2020). In their study, topographical global coherence increased with age in the delta and theta bands, and decreased in alpha. However, the authors used coherence as their connectivity measure, which is likely to involve artifactual coherence due to electromagnetic field spread. We employed the imaginary part of coherency, which has been reported to be less affected by field spread (Nolte et al., 2004). Our results on global connectivity confirm their findings in the theta and alpha bands, but also point to alterations with age in the gamma and beta band and additionally provide source information about these effects. Remarkably, the linear age effects on phase coupling were more local and mainly pronounced in the occipital lobe, with the frequency bands possibly exhibiting different but related functional processes. Marek et al. (2018) have reported theta-changes in phase-connectivity in the transition from adolescence to adulthood following an anterior-to-posterior gradient. According to their results and to another study covering the first five decades of life (Hunt et al., 2019), it is conceivable that frontal theta coupling decreases during this time period, while it slightly increases in posterior regions with age, as was observed in our analysis. Interestingly, the theta decoupling in the frontal cortex during adolescence was related to cognitive control (Marek et al., 2018). In general, theta oscillations are believed to temporally coordinate higher-frequency activity and to be fundamental to neuronal communication (Fries, 2005, 2015). Of note, in our study, gamma connectivity also increased significantly with age, showing a similar trend and spatial pattern as theta connectivity. Interestingly, in the primate brain, theta and gamma oscillations supported feedforward synchronization in visual cortical areas, whereas feedback communication relied on the beta band (Bastos et al., 2015). In the MEG Cam-CAN data set, others have observed that in these posterior brain regions covering

early visual areas, the occurrence of microstates decreased with age (Coquelet et al., 2020; Tibon et al., 2021). It is possible that these neural changes are related, for example, to the findings of reduced selective responsiveness of the ventral visual cortex to visual stimuli and slowing of perceptual speed in the elderly (Park 2004). In contrast, in higher-order areas, like frontotemporal and parietal regions, such transient neuronal states occurred more frequently with age (Tibon et al., 2021). These microstate profiles have been associated with lower fluid but not crystalline intelligence, which may indicate lower flexibility and coordination in the brain and overall lower neuronal efficiency (Tibon et al., 2021). In our study, frontocentral and temporal areas exhibited time-averaged connectivity following an inverted U-shaped pattern with age in the beta bands, with the highest connectivity levels in middle age and lower connectivity in the younger and older groups. In general, these higher-order areas may subserve intellectual, behavioral, and socioemotional processes and exhibit distributed long-range connections in the brain (Buckner & Krienen, 2013; Sepulcre et al., 2010; Sydnor et al., 2021). Consistently, beta oscillations have been linked to long-range brain communication before (Kopell et al., 2000) and our results may reflect developmental peaks around the 50s.
Our study supports the notion that connectivity in delta, theta, alpha, and gamma bands is likely to be relatively stable at a global level from early adulthood to old age, but exhibits local alterations, particularly in posterior brain areas. In contrast, strong nonlinear age effects on beta connectivity were observed in distributed centrotemporal and frontal areas. We extend electrophysiological studies in childhood or adolescence and show frequency-specific changes into old age.

Research on power alterations with age was undertaken early on with studies covering different life decades (Duffy et al., 1984). Overall, power decreases in lower frequency bands and increases in higher frequency bands with age have been consistently reported (Coquelet et al., 2020; Gómez et al., 2013; Hunt et al., 2019; Marek et al., 2018; Miskovic et al., 2015; Whitaker et al., 2016). We replicate previous results and add regional information for power redistribution across frequencies and distinct linear and nonlinear effects of age. Our data point to a delta power decrease in the cingulate and a U-shaped trajectory in the frontotemporal regions. Other electrophysiology studies have not examined the delta frequency band in this context (Coquelet et al., 2020; Hunt et al., 2019; Schäfer et al., 2014), and another study found no significant power differences across the lifespan in this frequency range (Sahoo et al., 2020). A possible explanation for this inconsistency with our findings could be a moderately lower reliability of power estimates in the delta range (Marquetand et al., 2019) and susceptibility to noise. Our results, however, tie in well with earlier work across the lifespan (Gómez et al., 2013), during the teenage years (Campbell & Feinberg, 2009) and early adulthood, showing delta band decreases in oscillatory activity along a posterior-anterior axis (Marek et al., 2018). Also in our study, frontal structures showed more of a delta decrease than posterior regions, which may be related to the development or decline of frontal cognitive functions. For example, decline in executive functions with age is thought to be related to structural changes in the frontal lobe (Greenwood, 2000; West, 2000). Consistently, a decrease in fluid intelligence and multitasking was found in the Cam-CAN data, to which changes in gray and white matter in specific prefrontal regions contributed (Kievit et al., 2014). Future studies should address this neurocognitive relationship in more detail, but the possible importance of delta oscillations for cognitive processes has been discussed previously (Harmony, 2013). Furthermore, the delta

power decrease in our study also affected cingulate and insular regions, which conversely exhibited a strong power increase in the beta and gamma frequency bands. Multiple, widely different functions in the brain are attributed to the insula (Nieuwenhuys, 2012), including interoception, for example, for pain, temperature, or tactile stimuli (Jones et al., 2010), as well as temporal and social perception (Schirmer et al., 2016). Insular dysfunction has been described in autism spectrum disorder, a neurodevelopmental disorder, and probably underlies several symptoms related to cognition, affection, and sensory functions (Nomi et al., 2019). Also, the insula is broadly connected to thalamic nuclei, amygdala, limbic, and association cortical areas (Nieuwenhuys, 2012). Remarkably, we also found power signals from the thalamus, putamen, and hippocampus to change over the lifespan in the beta1 and gamma bands. However, it is controversial whether MEG can reliably detect signals from deep structures, although one group has recently shown that it may be possible (Pizzo et al., 2019).
In addition, in our study, cingulate and central brain regions exhibited a quadratic effect of aging in the faster oscillations investigated, that is, high beta and gamma power. Cingulate regions are considered part of the DMN (Buckner et al., 2008) and to change during aging (Damoiseaux, 2017). The anterior cingulate cortex is assigned a central role in information processing and also a broad range of brain functions (Margulies et al., 2007), whereas the posterior part is thought to support internal cognition (Buckner et al., 2008; Raichle et al., 2001). Moreover, abnormal structure and function of the posterior cingulate cortex is associated with many neurological and psychiatric disorders with onset in adolescence and old age (Zhang & Raichle, 2010) and has been proposed to contribute to the metastability of intrinsic connectivity (Leech & Sharp, 2014). Central regions and beta band changes are classically related to movement (Jurkiewicz et al., 2006; Pfurtscheller & Neuper, 1997; Pfurtscheller et al., 1996). Interestingly, characteristics of movement-related amplitude alterations in beta, that is, during button pressing tasks, have been shown to change across the lifespan, as measured in the Cam-CAN data set (Bardouille et al., 2019). The findings were mainly restricted to primary somatosensory and motor areas, but also included activation of frontotemporal areas. It is conceivable that age-related changes in these networks during the resting-state over the lifespan, as observed in our study, lead to altered levels of event-related activity.
Altogether, power alterations in our study spatially encompassed the major players of the association network and the primary sensorimotor regions, with power decreasing at slower oscillations and increasing above 10 Hz. The reason for this redistribution needs further investigation, as does a possible relationship between phase-connectivity and power in beta frequencies. Currently, it is not clear why beta signals seem to be important for brain development and aging. One hypothesis is that beta oscillations may be involved in coordination within and between networks and, thus, hold a pivotal role across the lifespan (Briley et al., 2018).

We also explored whether males and females differ with regard to age-effects on MEG measures. Across the whole sample, there were no significant main effects of sex except for small frontal clusters in the beta2 and gamma bands (data not shown). Some studies report sex differences in MEG (Azanova et al., 2021; Fung et al., 2021; Hoshi & Shigihara, 2020; Taylor et al., 2020) or EEG features (Brenner et al., 1995; Clarke et al., 2001; Davidson et al., 1976; Kober & Neuper, 2011; Smit et al., 2008; Thordstein et al., 2006) during or in absence of a task in various age ranges and frequencies. However, research efforts in this direction for the resting-

state are generally limited. Our sample of 25 individuals of each sex per decade of life may not be sufficient to detect subtle main differences. However, there were significant interactions between sex and age in delta power and connectivity, and theta connectivity, while in the other frequency bands the global trajectories between the sexes were not significantly different. Looking at the distribution of global delta power in our data, we find that values are higher in males than in females in their early twenties and decrease more steeply with age. Decline in slow-wave activity during adolescence is known in the context of sleep research (Campbell et al., 2011; Campbell & Feinberg, 2009), with boys exhibiting more delta power than girls during the course, indicating an earlier onset of changes in sleep homeostasis in girls (Campbell et al., 2005). The occurrence of the "occipital delta of youth" is also a well-known EEG phenomenon that usually disappears with the transition to adulthood (Ebner et al., 2006), and could follow a different course in males and females, which would require further investigation. Similar to our results, Hunt et al. (2019) found less stable theta connectivity for males than females from childhood to middle age during rest, but alpha to gamma frequency were not affected. Another study reports sex-specific reconfigurations of EEG microstates during maturation and in the old age, pointing to varying trajectories of temporal dynamics for males and females (Tomescu et al., 2018). However, more studies are needed that systematically examine sex-dimorphic age-related patterns from an electrophysiological perspective and link them to other phenotypes to explore behavioral associations. Our study was balanced for age and sex, providing initial results suggesting that sex-specific differences in oscillatory lifetime dynamics can be expected in the slower frequency ranges.

Overall, we provide a comprehensive overview of changes in the characteristics of electrophysiological signals across the lifespan and complement age-related (f)MRI literature by resolving the spectral dynamics of the resting-state. We were able to model the relationship between age and connectivity or power by a linear function in some brain regions and a quadratic function in others. The Cam-CAN dataset used here does not cover the early years of life, which should be taken into account when interpreting our results. It is conceivable that the most extensive brain alterations occur in the childhood, adolescence, and early adulthood, when physical, cognitive and socio-emotional abilities are being formed, and then again in older age, when physical and cognitive decline occurs. White matter volumes have been found to increase into the middle adulthood, potentially due to extended myelination processes (Barnea-Goraly et al., 2005), whereas grey matter alterations were found to follow a differential pattern (Kochunov et al., 2011; Li et al., 2014). Structural underpinnings are likely to be intertwined with functional network behavior (Sadaghiani & Wirsich, 2020) and curvilinear associations between cortical volume and power changes with age have been reported (Whitford et al., 2007). However, the structure-function relationship across the lifespan is probably more complex. For example, we have found both functional increases and decreases with age in the same brain regions, depending on the respective frequency. With reference to fMRI findings, shifts in functional activation patterns in the elderly have been attributed to functional compensatory mechanisms (Davis et al., 2008). Others relate increased activity in the frontal lobe to decreases in neuronal efficiency or specificity (Morcom & Henson, 2018; Nyberg et al., 2012). However, without direct behavioral correlates, the interpretation of increased versus decreased power or connectivity during rest is challenging. Also, the relatively small effect sizes of the age effects on brain oscillatory characteristics suggest that many other factors

contribute to the variance in our data. Nonetheless, our results have important implications for a number of clinical studies using MEG or EEG in patients of different age and sex. When applying similar measures in clinical cohorts, convergence or divergence from the patterns in healthy individuals could be tested, primarily to understand brain pathology but also to advance the development of biological disease markers. However, one major limitation of our study must be kept in mind, namely its cross-sectional design. It is not suitable for determining dynamic long-term changes within an individual (Lindenberger et al., 2011) and cohort effects on our results cannot be excluded (Sliwinski et al., 2010). Longitudinal data would be most appropriate to study individual developmental processes, but the feasibility of such studies across the lifespan is unlikely with current techniques and methods.

To conclude, we demonstrate demarcated MEG phase-based connectivity and power patterns for primary and higher-order brain areas that are shown to develop differently across the lifespan. In addition, we provide evidence for sexually dimorphic trajectories in the lower frequency bands during rest. Our study can form a basis for further neurocognitive and clinical studies using electrophysiology.

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Disclosure

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4.4 THE RESULTS IN BRIEF

In study I, we showed that MEG power and connectivity are strongly increased in patients with GGE and, thus, provide evidence that network configurations are also altered during the interictal state. The connectivity increases for GGE were prominent in frontotemporal and central brain regions (in theta to gamma frequency bands), whereas power increases tended to be more widespread with a posterior focus, including occipital brain regions (in delta to gamma bands). Intriguingly, healthy siblings who had never epileptic seizures exhibited similar conditions; more precisely, the connectivity and power levels of the siblings were intermediate between those of the patients and those of the controls. We reported heritability estimates for MEG network measures, which were highest for the beta frequency band. In addition, connectivity was lower in patients with high drug exposure (AED) than patients with low exposure, especially in beta connectivity. Patients with generalized spike-wave discharges (GSWD) during the MEG recordings had higher delta connectivity than patients without GSWD, as well as higher delta and beta power.

The HD-EEG analysis in study II revealed increased network levels in GGE, similar to the MEG findings, but with spatial and spectral differences. HD-EEG power increases were more widespread and observed across the frequency spectrum (delta to gamma bands). Connectivity increases in the HD-EEG analysis were significant in the lower frequency bands, particularly in theta, whereas connectivity in the higher frequencies was not significant (beta to gamma bands), possibly related to medication effects. A statistical combination of HD-EEG and MEG maps yielded the strongest alterations in the theta and beta bands, suggesting that brain oscillations in these frequencies may be particularly relevant in GGE. Further, patients had reductions in cortical thickness, which corresponded spatially with the functional phenotype and enhanced the group contrast in a joint analysis. The healthy siblings exhibited similar HD-EEG and structural properties to the patients, but to a lesser extent.

Study III yielded different trajectories for power and connectivity across the lifespan. The linear and quadratic associations between age and the markers were spatially distinct, possibly mapping different developmental and aging processes. Global connectivity was relatively stable from early adulthood into old age but showed local linear changes in posterior regions (increases in theta and gamma, decreases in alpha to beta2 bands). In contrast, strong quadratic age effects on beta connectivity were found in central and frontotemporal areas (inverted U-shaped). Age effects on power spatially encompassed the major players of the association network and the primary sensorimotor regions, with power linearly decreasing with age at slower oscillations within the cingulate, and increasing above 10 Hz in insular, temporal, and central regions. Quadratic effects of age on power were observed anterior-basal (delta, theta; U-shaped), in the cingulate and central areas (high beta and gamma; inverted U-shaped). The lifespan trajectories for delta power and theta connectivity differed between males and females.

5 Discussion and Future Perspectives

This final chapter is intended to provide the reader with a conceptual and comprehensive treatise on electrophysiological networks in genetic generalized epilepsy (GGE) and further steps that lead to a promising interdisciplinary field of imaging, genetics, and human development. To begin with, the independent variables used as network markers in all three original studies are placed into context with other electrophysiological markers to guide their interpretation (Section 5.1). I then explain in more detail the significance of increased power and connectivity in GGE (Section 5.2), discuss the utility of these markers as endophenotypes, and what implications the studies in healthy siblings have (Section 5.3). Further, I aim to differentiate GGE from and elucidate features in common with other epilepsies (Section 5.4), and to discuss to what extent functional connectivity and power could be studied either alone or in a multimodal approach that would ultimately serve diagnostic purposes (Section 5.5). In addition, I address the question whether these markers change differently over the lifespan of patients with GGE compared with healthy individuals, and whether developmental or aging processes might contribute to the disease (Section 5.6). Finally, I offer suggestions on how electrophysiological data might be linked to the molecular make-up of the human brain (Section 5.7).

5.1 A word in advance about power and phase synchronization

Throughout this work, we focused on the imaginary part of coherency, a phase-based connectivity measure. Other coupling modes other than phase synchronization have been described, such as temporal correlation of signal amplitude, for example (Brookes et al., 2011; Bruns et al., 2000; Hipp et al., 2012). Very rapid fluctuations of neuronal oscillations in terms of burst-like activity could also coincide and coordinate networks (Baker et al., 2014), as has been found for the beta frequency in particular (Seedat et al., 2020). As outlined in Section 1.4.1, there are many available connectivity metrics (Bastos & Schoffelen, 2016) that quantify interdependencies between signals, each with their own advantages and disadvantages, and presumably also with different outcomes (Siems & Siegel, 2020). When using the imaginary part of coherency, an important caveat is that not only the phase of the signals but also the amplitude enters into the calculation (Nolte et al., 2004). Previous work has demonstrated that stronger phase-based connectivity in brain regions tends to be associated with a high signal power (Daffertshofer & van Wijk, 2011; Moon et al., 2015). Conversely, when the signal power decreases the signal-to-noise ratio (SNR) also decreases and the reliability of the phase estimate is reduced, as well. It follows that phase synchronization patterns should always be reported together with amplitude variations (Daffertshofer & van Wijk, 2011), as they could arise mainly on the basis of high signal power. We followed this recommendation in our analyses and found some overlap of significant differences in connectivity and power between the study groups in Chapters 4.1 and 4.2. However, there were also regional variations, which

argues against the possibility that our connectivity findings were solely due to variations in SNR. For example, the MEG analysis revealed strongly increased posterior power in patients with GGE, whereas the connectivity results were rather focused on frontotemporal regions. Also, as discussed in Chapter 4.3, the analysis of age-related differences in oscillatory features yielded spatially and temporally distinct power and connectivity trajectories. Nevertheless, other phase-based connectivity measures exist which could have been used instead. For example, one widely used measure is the phase locking value, which has been thought to reflect phase synchronization more strictly, since the amplitude normalized Fourier transforms across observations are included (Lachaux et al., 1999). On the other hand, it could be considered an advantage that the coherence metric leads to a better quality of phase estimation by giving more weight to observations with more stable signal strength and thus a higher SNR (Bastos & Schoffelen, 2016). Furthermore, it is argued that the coupling of oscillatory connectivity and amplitude is a natural condition of neural networks and should be expected to be observed in empirical data (Moon et al., 2015; Tewarie et al., 2019). Other exciting work has been initiated to further deepen the understanding of this relationship (Vidaurre et al., 2018).
In general, our results discussed in this dissertation should be interpreted in light of the selected imaging metrics. To validate our results, one could use other measures and see if they provided similar or different insights.

5.2 Increased functional network levels and their significance in GGE

Power and connectivity estimates indicated strongly increased levels in GGE during the interictal state (Chapters 4.1 and 4.2). However, the overall interpretation of this functional state and its role in ictogenesis is not trivial, but an attempt at an explanation will be made here. Brain oscillations reflect fluctuations of the membrane potentials of neural populations and, thus, timing of spiking activity and neural excitability (Fell & Axmacher, 2011) (Section 1.4.1). The alterations found in GGE therefore point to some loss of integration of postsynaptic potentials across neuronal assemblies. Classically, excitation of pyramidal neurons, which are considered the source of MEG and EEG signals, is assumed to be constrained by inhibitory interneurons and the neurotransmitter GABA (Buzsaki, 2006). In this way, a balance between excitation and inhibition is achieved, which, if disturbed, can lead to seizures in otherwise normal brain tissue and marked oscillatory changes (Scharfman, 2007; Staley, 2015). Conversely, seizure activity can be blocked by pharmacologically increasing inhibition (Wiechert & Herbst, 1966) or suppressing excitation (Croucher et al., 1982). Thus, as in other epilepsies (Section 1.1.5), impaired inhibition may underlie GGE. This is supported by several findings. For instance, genetic variations in GABA receptors were associated with GGE (Section 1.2.2). Further, the mechanisms of action of many antiepileptic drugs (AED), which are commonly used in GGE, focus on enhancing GABA transmission, whereas others act on voltage-gated sodium or calcium channels (Devinsky et al., 2018). In addition, higher cortical excitability has been reported in patients with GGE compared with controls (Badawy et al.,

2007; Badawy et al., 2013), possibly indicating disturbed inhibitory circuits. This is, however, probably not the complete picture.

It also raises the question of what role different frequencies might play in the altered inhibition processes in GGE. Different brain rhythms are thought to coexist and together orchestrate different brain states, with inhibition providing the basis of rhythmic activity (Buzsaki, 2006). In our MEG-and HD-EEG analyses of power, all frequency bands studied (delta to low gamma) were affected, potentially indicating a temporally independent, distorted inhibition in GGE. Connectivity changes, on the other hand, were prominent in the theta (~6 Hz) and low beta (~16 Hz) bands (Chapters 4.1 and 4.2). Theta oscillations play a critical role in the transition to seizure activity in mice by affecting the function of interneurons (Moxon et al., 2019). Moxon et al. (2019) proposed that theta oscillations and particularly hyper-synchronization rather than simple hyper-excitability in this frequency range could modulate seizures and presumably also cognitive control. Also, in primates, low-frequency oscillations have been shown to align spatiotemporal dynamics by phase resetting and presumably triggering coherent activity in the brain (Daitch et al., 2013; Voloh et al., 2015). This mechanism could facilitate accurate shifts in attention or other behavioral tasks and essentially control the timing of brain activity (Daitch et al., 2013; Voloh et al., 2015). Whether theta oscillations during the resting-state in humans might be a kind of control or coordination instance, also with regard to the emergence of seizures from the interictal state, needs further investigation. One could also speculate whether theta oscillations during rest interact with higher frequency activity in epilepsy, as has been previously reported in context with cognition (Lisman & Jensen, 2013). For example, in the MEG analysis, increased connectivity was also observed in low gamma, which is often related to more local processing in the brain (Buzsáki & Wang, 2012). Beta connectivity also seems to play a significant role in GGE as discussed in Chapter 4.1. Interestingly, a direct relationship between GABA concentrations and beta peak frequencies at rest (Baumgarten et al., 2016) and beta power during movement has been found in the sensorimotor cortex (Gaetz et al., 2011; Muthukumaraswamy et al., 2013). Parallel to a central function in movement-related processes, beta oscillations are involved in long-range connectivity in the cortex (Brookes et al., 2011; Engel et al., 2013; Hipp et al., 2012). But how can increased connectivity, in our case phase synchronization, be interpreted? It is currently unknown how inhibitory modulation of signal amplitude ultimately drives connectivity in the brain (Seedat et al., 2020). Correlated neuronal activity is thought to facilitate communication between different brain areas (Singer, 1999). Brain regions in phase, that is, coherent, could provide windows for flexible and optimal information transmission through action potentials (Fries, 2005, 2015; Varela et al., 2001). It has been shown that highly connected brain areas also exhibit phase-coupling in the resting-state (Engel et al., 2013; Vidaurre et al., 2018). In contrast, recent work proposed that coherence does not reflect the cause but the consequence of inter-areal communication in the brain (Schneider et al., 2021). As such, the spiking activity in cortical neurons would not only trigger synaptic potentials in the neighborhood but also in more distant, connected cells with a delay. Accordingly, rhythmic activity in a sending brain region would naturally induce coherence in local rhythms of the receiver (Schneider et al., 2021). Based on these two hypotheses, increased connectivity could

be an expression of increased information exchange, or something like a control mechanism of one brain area over another based on their power profiles and anatomical connections. Interestingly, in our cohort, EEG connectivity was associated with cognitive performance, as shown by preliminary results (Other Contributions). High network synchrony in frequency bands where patients significantly differed from controls was related to weaker attention and problem-solving ability. In turn, cognitive performance was reported to be impacted by the occurrence of interictal epileptic discharges (Rausch et al., 1978; Ung et al., 2017), supporting a neurobehavioral link.

But does it follow that increased macroscale power and connectivity promote the occurrence of epileptiform discharges or seizures in GGE, maybe through long-range connections? A disturbed inhibition in the brain may map to, for instance, an attempt to restore a balanced level of inhibition as a sort of compensation, or reflect an altered sequence of excitation and inhibition leading to seizures. In the first case, an evolvement of a seizure from an interictal state with strong power and connectivity would be less likely. Conversely, should increased network power and connectivity be some sort of precursor to seizures, the occurrence of seizures from this state would become more likely. Given the varying levels in the network metrics with drug exposure (reduced connectivity) or epileptic discharges (increased power and connectivity) in our GGE cohort, the latter hypothesis seems more plausible at a first glance. Some have argued that epileptic discharges are related to deviations from critical dynamics, hence the brain state would be closer to neuronal runaway activity (Arviv et al., 2016). However, others have observed that epileptiform discharges produce phasic changes in a network, either facilitating (Huberfeld et al., 2011) or preventing seizures (Karoly et al., 2016) or both (Chang et al., 2018). Interestingly, in the epileptogenic zone in patients with focal epilepsies, the pre-ictal phase was characterized by spiking activity and also by suppression of delta to low gamma activity coupled with the occurrence of rapid high-frequency activity at seizure onset (Grinenko et al., 2018). The authors also showed that during the transition to the ictal state, fast inhibitory neurons were acting on or suppressing activity from pyramidal neurons at the seizure onset (Grinenko et al., 2018). That study does not help to clarify the role of epileptic discharges or increased interictal networks, but does demonstrate the involvement of relatively low-frequency activity around seizure onset and reveals the complex interplay of these processes.

Many electrophysiology micro- and macroscale studies have so far focused on network dynamics during or in close temporal proximity to ictal states (Section 1.2.4) rather than on longer interictal phases, which is the most frequent state in patients with GGE. We examined five minutes of cleaned interictal data and provided evidence that altered network states are generally present or may be changing rapidly and recursively even in those patients who did not exhibit observable GSWD during EEG/MEG recordings. Our results further suggest the involvement of frontotemporal, central, and occipital brain regions in GGE. To some extent, this is consistent with the results of resting-state fMRI studies that show connectivity changes primarily in the default mode network (DMN) (Section 1.2.4) encompassing some of the observed regions. This may not be surprising given that the electrophysiological connectome appears to correspond to fMRI resting-state networks (Brookes et al., 2011; Hipp & Siegel,

2015; Sadaghiani & Wirsich, 2020), especially when canonical frequencies are considered together (Tewarie et al., 2016). Interestingly, using MEG rapid reconfigurations (~50-100 ms) were observed in the DMN, sensorimotor, and visual networks, revealing independent anterior and posterior functional parts of the DMN at certain frequencies (Vidaurre et al., 2018).

Although it is not possible to fully elucidate the nuanced aspects of increased connectivity and power in GGE, the findings discussed above and the results of our work have substantial added value. They highlight the unique opportunities that the high temporal resolution of MEG and EEG, as well as power- and phase-based markers, offers to unravel the mechanisms of network interactions in future studies. A more detailed investigation of dynamic network changes in the resting-state could provide a sophisticated picture of dysfunction in GGE and clarify the role of temporal hierarchies such as theta and beta frequencies.

5.3 Similar network phenotypes in healthy siblings

In Chapters 4.1 and 4.2, I present the observation that siblings of patients, who never experienced epileptic seizures, also have increased MEG and EEG power and connectivity compared with controls. Thus, this network state in GGE does not merely reflect disease progression or consequences of secondary disease effects, but rather indicates a genetic influence, which has important implications. First, the electrophysiological markers formally meet the suggested criteria for an endophenotype marker (Gottesman & Gould, 2003) (Section 1.3.1). In specific, increased power and connectivity co-segregated in unaffected family members of the patients, but were also associated with the illness. This demonstrates the unexplored potential of using oscillatory markers to discover genetic mechanisms in GGE, as will be discussed in more detail in Section 5.7. Second, our findings in siblings indicate that there is probably no “standard” or “pathological” power or connectivity network state per se, so that a categorical distinction between healthy and diseased based on network levels by themselves seems difficult. In all three studies included in this thesis, a large interindividual variability stands out despite robust statistical group differences, suggesting that neurophysiological activity in humans varies considerably between individuals, which is a common observation in many studies (Holmes & Patrick, 2018). In Chapters 4.1 and 4.2, we present the data distributions of global power and connectivity in the patients, siblings, and controls, and find a clear overlap between the individuals of the different groups. Simply put, a higher network connectivity or power is not necessarily always pathological if the network is studied in isolation. In the same vein, a variation in cortical thickness likely does not necessarily imply an adverse (behavioral) outcome, if our results in siblings (Chapter 4.2) can be confirmed in studies with a larger number of participants. Also, familial and environmental factors may have contributed to the similarity in the network traits between family members. Yet, in our study, connectivity patterns were associated with disease aspects, that is, GSWD in patients. Also, one of the 18 investigated siblings had GSWD during the EEG recording without a history of seizures or epilepsy. An increased prevalence of interictal epileptiform discharges in siblings is known (Atakli et al., 2000; Doose et al., 1977), but they also occur in

approximately one to five percent of the general population (Gregory et al., 1993; So, 2010). This may relate to the higher risk for GGE in first-degree relatives and to the fact that single spontaneous seizures can arise in healthy individuals without developing epilepsy with recurrent seizures. It is possible that the formation of a balance between excitation and inhibition in the brain is genetically imprinted and that we have recorded the individual level of this balance with electrophysiological measurements. These levels may eventually translate into seizure susceptibility, perhaps due to experience, environmental influences, or other (epi-)genetic factors. Finally, the question remains as to what causes molecular pathways to go off track and cause chronic GGE in some people but not in others. Potentially, a set of multivariate "fingerprints" that includes genetics, brain and behavioral characteristics of an entire individual, and ideally the environmental context, would help improve our understanding of susceptibility to disease (Holmes & Patrick, 2018). In fact, individual characterization using imaging markers seems possible. Robust differentiation of individuals has been successful based on cortical structure (Tian et al., 2021), but also on fMRI brain function and was linked to behavioral measures (Finn et al., 2015; Tian et al., 2021). With some regional variability, accurate and robust fingerprints for MEG resting-state power spectra and the connectome have also been described (Da Silva Castanheira et al., 2021; Sareen et al., 2021). Previous EEG studies had already reported individual characteristic features (Demuru & Fraschini, 2020; Fraschini et al., 2014; Kong et al., 2019), but were limited to the sensor level.
Taken together, increased network power and connectivity may signify genetically influenced mechanisms in GEE. At the same time, our data implies that these electrophysiological markers are expressed on a continuum and that an individual risk of disease can probably only be determined if individual phenotyping of multiple traits is taken into account.

5.4 Functional and genetic overlap with other epilepsies and within GGE subtypes

If increased power and connectivity represent compensatory mechanisms or a precursor state of a seizure, one might anticipate similar network states in different forms of epilepsy. However, regional differences or variations in the spatial extent of increased activity or connectivity are to be expected, for example, more local patterns in focal epilepsy than in generalized epilepsy. In fact, increased MEG connectivity was also found in a group of cryptogenic / non-lesional focal epilepsies, albeit with spatial differences in comparison to GGE patients (Li Hegner et al., 2018). Even though medial frontal and sensorimotor areas were more strongly affected in GGE, this finding point to common network alterations in the various epilepsy types. Also, earlier studies have described increased functional connectivity in the epileptogenic zone of focal epilepsy patients (Bettus et al., 2008; Wu et al., 2014). In general, despite the remarkable symptomatic and pathogenic heterogeneity of the epilepsies, there are common functional and structural alterations that may also arise from common genetic mechanisms. Previously, some genetic association signals related to the *SCN1A*, *SCN2A*, and *SCN3A* genes have been found to overlap in focal and genetic generalized epilepsies (The International League Against Epilepsy, 2018). Interestingly, mutations in all three genes cause

ion channel dysfunction and are an established monogenic cause of epileptic encephalopathies (The International League Against Epilepsy, 2018). Whether variants in these genes lead to an imbalance between excitation and inhibition in focal epilepsies and GGE in a similar manner as suspected for the encephalopathies, however, is an unanswered question. Other common genetic susceptibilities have been identified for monogenic epilepsies and complex GGE. Mutations in the *STX1B* gene were reported to cause fever-associated epilepsies of varying severity ranging from simple febrile seizures to developmental and epileptic encephalopathies (Schubert et al., 2014; Wolking et al., 2019). A strong genetic signal for *STX1B* was also detected in JME, the largest GGE subtype. *STX1B* encodes syntaxin-1B, a presynaptic protein which is part of the SNARE complex that mediates presynaptic vesicle release (Smirnova et al., 1996). Given this genetic and phenotypic spectrum of rare monogenic and common complex epilepsies, it may be informative to map functional brain networks in patients of both groups and compare them with their healthy relatives. First, this comparison could reveal mechanistic similarities and differences between epilepsies. For example, one could clarify whether increased power and connectivity reflect an overriding susceptibility to increased network excitability or other factors typical of GGE. Second, imaging findings in monogenic epilepsies with a known genetic cause could be better linked to molecular mechanisms that may involve whole brain networks. A major limitation, however, is that these patients are relatively rare, and collecting data from them and their families requires collaboration among multiple hospitals and research centers.

In this context, it is also worth mentioning the subtypes of GGE, which are also thought to share significant genetic susceptibility (Smirnova et al., 1996) with presumably specific determining effects. As early as the 1980/1990s, a neurobiological continuum for GGEs was proposed rather than assuming distinct subtypes, a concept that is supported to some extent by current genetic findings (Berkovic et al., 1987; Reutens & Berkovic, 1995). This is also in line with the semiological presentation of patients, which is often inconclusive (Section 1.2.1), and with studies examining cognition or other imaging modalities, most of which have revealed subtle differences between subtypes (Ratcliffe et al., 2020; Vorderwülbecke et al., 2021). The size of our study sample did not enable us to discern network effects for single GGE subtypes, but they contributed relatively equally to the overall GGE sample (Chapters 4.1 and 4.2). However, about 20 % of the patients could not be clearly assigned to one of the four classic subtypes, which corresponds to the general observation in clinical routine (Vorderwülbecke et al., 2021). Further studies would, therefore, be needed to disentangle subtype specific patterns.

From an imaging, genetic and clinical perspective, it seems evident that certain forms of epilepsy blend into each other. This could challenge the discrete delineations between epilepsies based on electrophysiological markers for clinical diagnosis and treatment. Conversely, studies of different epilepsy syndromes, particularly those with a clear biological etiology, could help solidify the validity and specificity of imaging markers such as power and connectivity.

5.5 (MULTIMODAL) IMAGING AND DIAGNOSTICS IN GGE

The question arises whether resting-state power and connectivity could be of support in clinical routine or diagnostic processes in the future. As stated in the introduction (Section 1.1.3), the current diagnostic criteria for epilepsy are based on descriptive guidelines and, in the first instance, mostly on clinical observation and self-reports by the patient. Usually, an EEG recording or cranial imaging is used additionally. In this context, great hopes are pinned on technical developments in the field of computer science or even molecular genetics. For example, machine learning and the accumulation of large data sets will be used to improve algorithms through data rather than by explicit instructions (Abbasi & Goldenholz, 2019). For GGE, a few studies have already implemented such approaches, for example, for automated detection of epileptiform events in the EEG (Clarke et al., 2021) or for discrimination of JME from controls based on diffusion MRI properties (Lee et al., 2021). In other studies, MEG data at the sensor level in the resting-state were used to discriminate healthy subjects from patients with GGE or frontal focal epilepsy (Soriano et al., 2017) or from patients with JME (Lopes et al., 2021). Interestingly, Soriano et al. (2017) report that relative MEG power was sufficient to discriminate controls from epilepsy patients with high predictive accuracy, whereas only a combination of power and connectivity features allowed discrimination between GGE and focal epilepsy, particularly in the beta frequencies. It should be noted, however, that in all of the above-mentioned studies, the size of the data sets was limited. Small sample sizes may increase the risk of overfitting and inadequate treatment of outliers in the data (Lemm et al., 2011), and it is possible that clinical variables may confound the results (Abbasi & Goldenholz, 2019). In general, this calls for large, coordinated data sets and external validation of results, as well as work on other technical barriers to enable translation of biomarkers to clinical practice in the future. The comparatively strong effect sizes for HD-EEG and MEG group differences in our work (Cohen's *d* up to 1.3) also encourage further efforts in this direction. Moreover, we and others have demonstrated that EEG/MEG signal power can be very reliable with as little as about one minute of clean data (Gasser et al., 1985; Lew et al., 2021; Salinsky et al., 1991; Van Albada et al., 2007), both at the global level and in most brain regions (Marquetand et al., 2019), and that it is a very stable marker even with about 30 seconds of data (Wiesman et al., 2021). Reliability for the imaginary part of coherency using MEG and HD-EEG was lower than for power especially for delta band EEG, which requires longer recordings overall (Marquetand et al., 2019). Also, phase-based connectivity has been less reliable than amplitude-based measures (Colclough et al., 2017; Duan et al., 2021; Rolle et al., 2021), which would argue in favor of using the latter in translational studies. However, it has been shown that those two modes at least partly reflect distinct mechanisms in the brain (Mostame & Sadaghiani, 2020; Siems & Siegel, 2020).

Network science has taught us that changes in one part of the brain usually occur together with other disturbances in the system (Bassett & Sporns, 2017; Buckholtz & Meyer-Lindenberg, 2012). Although not always easy to implement technically, multimodal approaches can broaden the perspective on a disease or the interaction of multiple biological levels and support (differential) diagnostics by integrating different features. As discussed in

Chapter 4.2, the joint use of HD-EEG and MEG yielded complementary information in GGE at the group level. At the individual level, this combination has also been used successfully, that is, in the presurgical setting for intractable focal epilepsies (Rampp et al., 2019). Some also report that interictal epileptiform events were detectable in both MEG and EEG, but sometimes only in one of the two techniques (Lin et al., 2003; Ossenblok et al., 2007; Scheler et al., 2007). Further, we provided evidence that the inclusion of cortical thickness reductions corresponded to functional patterns in GGE. This underlines the notion of a relation between brain rhythms and anatomy. For example, Mahjoory et al. (2020) have demonstrated a spatial gradient for MEG oscillations anticorrelated with that of cortical thickness. Others have shown in monkeys that connectivity networks at different frequencies may depend on anatomical architecture (Vezoli et al., 2021). Our data suggest that structural and functional features should be jointly used to more precisely characterize GGE. However, we must acknowledge that our connectivity estimates are based on pairwise connections, and likely to inaccurately map the topographical interactions in the brain (Smith et al., 2011). Therefore, another way to improve the estimation of functional connectivity and discrimination at the group-level could be to consider anatomical connectivity (Deco et al., 2013; Haimovici et al., 2013). Such an approach of structurally guided functional estimation has been successful earlier in clinical samples (Pineda-Pardo et al., 2014) and healthy individuals (Finger et al., 2016). Clearly, more work needs to be done to understand how best to combine different modalities to actually increase the diagnostic potential of imaging markers. For GGE, we have taken initial steps to bring together functional and structural features at the population level.

Overall, collaborative (multimodal) imaging efforts can help advance a biologically oriented classification of GGE alongside clinical observation. In this regard, versatile electrophysiology will contribute well, by including resting-state measurements that can be easily incorporated in the clinical setting. Precision medicine for epilepsy, including GGE, would be the ultimate goal to enable early diagnosis and therapy, reduce disease burden, and predict seizure occurrence and disease activity. As alluded to in Section 5.3, this is likely to require phenotyping based on a combination of electroclinical imaging, pharmaco-response, and genetic building blocks (Thakran et al., 2020), and will depend heavily on the availability of the appropriate techniques.

5.6 Electrophysiology across the lifespan with reference to GGE

When using electrophysiological markers for diagnostic purposes or in clinical studies, it is essential to know the factors that influence the expression of these markers. In Chapter 4.3, we have shown that power and phase-based connectivity vary across the lifespan and probably map developmental and aging processes. On the one hand, this emphasizes the importance of considering the age of the patients when assessing EEG (or MEG) recordings in the clinical routine and of aiming for balanced study designs to avoid age-related biases. Moreover, this motivates further questions related to GGE: do patients with GGE deviate from normative electrophysiological trajectories across the lifespan? Do developmental and age-related processes play a role in GGE, as assumed (Section 1.3.3)? Also, the “brain age”

approach presented earlier could reveal more about the state of the brain at a given time in a (diseased) person (Section 1.3.3). The following is a discussion of these questions with reference to our data.

In our work, the age distribution in the GGE and control samples ranged from 18 to 50 years and was matched for the groups. In the Appendix, we provide an overview and statistical analyses for the expression of global power and connectivity with age for GGE and healthy controls. We found significant interaction effects between study group and global connectivity with age in the EEG theta band and the MEG gamma band. For power and for other frequency bands, the global trajectories of the patients roughly paralleled those of the controls. Theta connectivity as measured by EEG is likely to play an important role in GGE (Chapter 4.2). MEG gamma connectivity was also significantly increased in the patients (Chapter 4.3). An interaction effect in these frequency bands could, thus, be an indication of meaningful age-related mechanisms in GGE. However, the data density is skewed and sparse at the upper end of the age distribution, clearly compromising the comparability of the slopes between the groups. Therefore, an estimation of the development of power and connectivity with age in GGE with our data should be interpreted with caution. It should also be mentioned again that GGE usually arises in childhood or early adulthood (and in some cases also in late adulthood). The patients in our sample recruited in an adult epileptology clinic were all older than 18 years, with a median age at onset of 15 years (interquartile range 10-17).

Interestingly, Vidal-Pineiro et al. (2021) suggested that the individual variation of cross-sectional brain age rather reflects early life influences or genetic underpinnings than accelerated brain aging as demonstrated for MRI features (Section 1.3.3). It should be tested whether this holds true in a similar way for electrophysiological brain age estimations. However, this hypothesis implies that deviation from the norm does not increase gradually with age, but that there might be a specific time in life when an initial deviation occurs and then develops stably along the normative aging trajectory. In concrete terms for GGE, this could mean that relative increases in connectivity and power occur in the short term or gradually early in life, develop at this level with age, and are eventually accompanied by seizures. Since the healthy siblings also had higher levels of connectivity and power than the controls in our studies, it is possible that these electrophysiological traits are expressed early in life and transition to a state of seizure susceptibility and chronic epilepsy at a certain point or over some period of time. In addition, the duration of GGE could also have an impact on interictal oscillatory changes with age, which has not yet been adequately investigated. However, if increased power and connectivity reflect seizure susceptibility, this scenario seems improbable as the likelihood of seizures in adults with GGE tends to decrease with age (Vorderwülbecke et al., 2021). Also, in our MEG analysis (Chapter 4.1), there was no effect of epilepsy duration after controlling for age, and the sub-analyses for EEG did not allow a clear separation from other variables such as sex effects (Chapter 4.2).

Of note, age at epilepsy onset may cluster within families (Kinirons et al., 2008; Tsuboi & Endo, 1991). A recent study has shown that this is likely partly independent of the different epilepsy syndromes and subtypes, that is, family members have comparable age at seizure onset due to familial aggregation of a syndrome but also because of family membership (Ellis et al.,

2019). The authors suggested that this could indicate distinct genetic determinants that may be involved in the time course of neurodevelopment and interact with causal genetic factors of epilepsy. As such, the timing and its genetic basis seem to play an intriguing role alongside disease susceptibility per se. This has similarly been described for schizophrenia (Hare et al., 2010), where the first symptoms usually emerge in the mid-twenties (about a few years later for females) (Häfner et al., 1993). Interestingly, extensive reorganization of genes at the time of symptom expression has been demonstrated, essentially controlling the onset of the disease (Skene et al., 2017).

Clearly, longitudinal studies would be needed to best track network changes in GGE, particularly around disease onset, but this poses a methodological challenge. The studies of electrophysiological markers in our GGE cohort and in healthy individuals encourage further efforts to map such changes across the lifespan. In particular, the early years in childhood up to adulthood of patients with GGE and, at best, the period around the first seizure could be informative. Further, genetic underpinnings of electrophysiological markers across the lifespan should be deciphered. It seems valid to assume that genetic factors for GGE also converge on biological pathways influencing functional brain development.

5.7 Implications for linking electrophysiology and genetics

In this final section, I will take a broader look at how electrophysiological markers can help elucidate molecular mechanisms in GGE or changes across the lifespan. As discussed above (Section 5.3), power and connectivity can be used as endophenotypes for GGE, following examples of previous work in psychiatric research. Rather than performing genome-wide association studies (GWAS) for cases and controls (Section 1.2.2), which require enormous sample sizes, it might be useful to focus on disease components such as electrophysiological patterns. The advent of large population-based cohorts can allow such investigations, as has been demonstrated in a number of papers in recent years. For example, the behavioral trait "impulsivity" has been shown to be heritable and genetically correlated with ADHD and substance use disorders (Sanchez-Roige et al., 2019; Sanchez-Roige et al., 2018). In another case, GWAS on executive functions (Ibrahim-Verbaas et al., 2016), risk tolerance (Linnér et al., 2019) and sensation seeking (Sanchez-Roige et al., 2019) revealed a locus encompassing the gene *CAMD2*, which was also associated with alcohol use disorder (Kranzler et al., 2019). Others have used the large-scale UK Biobank for a GWAS of MRI phenotypes and identified genes relevant for brain development and plasticity, ion transport, nutrients and minerals (Elliott et al., 2018). In this analysis, more than 3,000 image phenotypes derived from grey matter features, white matter connections, and resting-state fMRI were studied. This selection of studies underscores, on the one hand, that neurobiological insights into disease-related bases can be gained in this way, but on the other hand, that the notion of a simpler genetic architecture of endophenotypes is not readily tenable (Sanchez-Roige et al., 2019). Accordingly, a polygenic basis should generally be expected for endophenotype models.

To date, similar efforts have been rare for electrophysiological markers, although EEG traits are highly heritable (Smit et al., 2005; Tang et al., 2007; Van Beijsterveldt et al., 1996; Zietsch

et al., 2007). In 2018, Smit and colleagues published first results of the largest GWAS to date on EEG power in the delta to beta frequencies (Smit et al., 2018). One prominent finding was an association of beta power with hippocampal expression of the *GABRA2* gene (Smit et al., 2018). Interestingly, *GABRA2* seems to be an important risk gene for GGE (The International League Against Epilepsy, 2018). The link between high beta power and genetic GGE risk was later confirmed (Stevelink et al., 2021). In addition, the authors reported that the tendency to increased theta power was also associated with higher GGE risk. These findings fit very well with our own study results and support the conceptual validity of an endophenotypic approach in GGE. We complement these large-scale studies with regional information and the observation that heritability appears to be strongest in the beta and theta bands but may extend to other frequency bands. However, larger sample sizes in clinical studies would be needed to confirm the latter. In the presented EEG-GWAS study (Smit et al., 2018), the signal was sampled at only three electrodes, so there is still room for improvement of spatial and temporal resolution in further efforts.

One issue with large statistical association analyses of imaging phenotypes and genomic data is that the functional role of identified variants is often unknown. Follow-up analyses, for example in animal models, are needed to understand how such a gene variant would affect the phenotypic variation. Also, gene expression levels mostly vary across brain regions (Hawrylycz et al., 2012), something which cannot be assessed from sequencing alone. The availability of brain-wide gene expression atlases of post mortem brains of humans or other species have opened exciting new opportunities to link molecular function and imaging data. One of the most comprehensive atlases today is the Allen Human Brain Atlas (Hawrylycz et al., 2012). It contains measurements of the transcriptional activity of over 20,000 genes at several thousand anatomical sites in six different post mortem human brains. Spatial mapping using coordinates makes it possible to relate the atlas gene samples to functional imaging patterns in a study population (Arnatkevičiūtė et al., 2019). Finally, in principle, the relationship between spatial variations in gene expression with variations in imaging-derived patterns is tested statistically. In addition, associated genes can also be tested for their presumed function by examining their enrichment in ontological databases (Arnatkevičiūtė et al., 2019). Of course, these methods are also subject to methodological caveats and a unified approach is proposed (Arnatkevičiūtė et al., 2019). Nevertheless, this approach has already led to informative findings. Richiardi et al. (2015) and Wang et al. (2015) were among the first to directly correlate transcriptional gene activity with fMRI resting-state networks, finding gene sets associated with ion channel activity and synaptic function, among others.

Returning to the results of our work, given the relatively high heritability of GGE and electrophysiological markers, their use as endophenotypes could find application in large-scale GWAS studies, or could directly yield new insights with the available genetics data. This ultimately concerns the pathophysiology of GGE, but also developmental and aging processes, which in turn play a significant role in disease.

5.8 Conclusion

This work significantly contributes to a broader understanding of large-scale network alterations in GGE by demonstrating widespread increases in multiple frequencies during the resting-state. MEG/EEG power and connectivity metrics differently characterized this network state in GGE involving frontotemporal, central, and posterior brain areas, potentially reflecting a precursor state to seizures or compensation. Our studies in healthy siblings indicate a genetic influence on this state rather than effects attributable to disease progression or treatment, highlighting the potential for the use of electrophysiological markers in genetic studies. It could be shown that cortical thickness complements the functional phenotype of GGE, encouraging multimodal approaches to better understand brain changes in GGE. This approach, together with comparative studies in other types of epilepsy, may help to improve the previously descriptive, clinically oriented diagnosis based on imaging findings. The work described here further elucidates changes in power and connectivity across age, which should be taken into account when applying these markers in clinical studies or the diagnostic context. Furthermore, variations in these markers, their genetic basis, and behavioral correlates across the lifespan should be explored to relate them to disease development in epilepsy.

6 Other Contributions

6.1 Cognitive profiles are linked to EEG phenotypes in patients with genetic generalized epilepsy

Authors: Christina Stier, Markus Loose, Niels K. Focke

My contributions: Conductance of neuropsychological examinations, documentation and scoring. Test scores and group differences were statistically evaluated in collaboration with a medical doctoral student under my supervision and further related the functional alterations in patients with GGE.

Unlike many other epilepsy syndromes, genetic generalized epilepsy (GGE) is associated with comparatively mild cognitive impairments (Ratcliffe et al., 2020). In general, patients' IQ scores are usually within the normal range (Loughman et al., 2014; Ratcliffe et al., 2020), but poorer academic performance and psychosocial outcome have been noted (Camfield & Camfield, 2009; Guida et al., 2019; Shakeshaft et al., 2021). Executive functions often appear to be affected (Loughman et al., 2014; Ratcliffe et al., 2020), including working memory (Chowdhury, Elwes, et al., 2014; Vollmar et al., 2011), prospective memory (Wandschneider et al., 2010) such as planning and implementation of intentions, as well as linguistic abilities (Chowdhury, Elwes, et al., 2014; Iqbal et al., 2015) and attention (Chowdhury, Elwes, et al., 2014). Moreover, pharmacological treatment is expected to affect cognitive abilities, making it challenging to separate causal effects from secondary treatment effects. For example, sedation (Mattson et al., 1985), cognitive impairment (Mattson et al., 1985), loss of concentration (Bresnahan et al., 2019), and neuropsychiatric adverse effects (Chen et al., 2017) may occur with common treatment options in GGE.
The extent to which cognitive changes are related to interictal electrophysiological patterns in patients with GGE has not yet been investigated. We associated global HD-EEG power and connectivity with the cognitive profiles of the patients in our GGE sample. In specific, we tested attention, concentration, and speed (d2-R Aufmerksamkeits-Belastungstest; Brickenkamp et al. (2010)), auditory verbal learning (VLMT Verbaler Lern- und Merkfähigkeitstest; Helmstaedter and Durwen (1990)), figural memory (DCS-II Diagnosticum für Cerebralschädigung II; Weidlich et al. (2011)), working memory (Wechsler's digit span test; Coalson et al. (2008)), psychomotor speed and cognitive flexibility (TMT Trail Making Test; Reitan and Wolfson (1995)), and problem solving (Tower of London; Phillips (1999)). All patients were assessed for symptoms of depression (BDI-2 The Beck Depression Inventory Beck et al. (1996)) and premorbid (crystallized) intelligence (MWT-B Mehrfachwahl-Wortschatz Intelligenztest; Lehrl (2005)). The test results for 19 patients were available for further analysis. Analyses were performed using linear models with the neuropsychological test scores as dependent variables. Global HD-EEG power or connectivity were the independent variables, as were drug exposure (number of AEDs), age, and sex.

Overall, higher connectivity and power in lower frequency bands (delta, theta) were significantly associated with worse performance in attention and problem-solving domains. As discussed earlier (Chapter 4.2), comparing the HD-EEG metrics with those of the control cohort showed that increased values in these frequency bands are characteristic of GGE and probably indicative of pathological mechanisms. Thus, it is plausible that lower attention and reduced problem-solving ability may be directly related to electrophysiological patterns typical of GGE. Conversely, higher connectivity in the higher frequencies (beta1, beta2, gamma) was associated with better verbal and working memory scores. Remarkably, at the group level, patients with GGE did not statistically differ from controls in connectivity in these higher frequencies (Chapter 4.2), suggesting that network markers and cognitive performance were in the normative range. For instance, it could be that increased network synchrony is generally linked with better performance in these cognitive domains in a healthy population. Further, the analyses indicated positive medication effects on verbal memory, yielding better scores for patients with high drug load than patients taking fewer drugs. Consistently, HD-EEG connectivity was lower in patients with high drug load, falling within the range of healthy controls in higher frequencies (Chapter 4.2). At the same time, medication load was negatively associated with the performance in the trail making test, which assesses psychomotor speed, and the D2 attention task in the patients. In line with earlier work (Meador et al., 2001; Witt et al., 2015), antiepileptic medication may have reduced the processing speed in the patients studied and thus, task performance. The relationship between adverse effects of medication on cognitive performance needs to be further studied with a repeated measures design, before and after the medication intake. However, this is a first attempt to disentangle the interplay between electrophysiological patterns and cognitive functioning in GGE, encouraging future neurobehavioral studies. We provide evidence for a negative association of increased HD-EEG power and connectivity with attention and problem-solving skills in GGE. Performance in the verbal and working memory of the patients was positively associated with connectivity in higher frequencies, which was within the range of controls and probably related in part to medication effects.

APPENDIX

NETWORK PHENOTYPES IN GGE ACROSS AGE

As a supplement to the discussion in Section 5.6, an overview is provided of how power and connectivity differed across age in patients with GGE and controls in the study cohort presented in Chapter 4.2. The linear trajectories of MEG gamma connectivity (Figure 7.1.1) and EEG theta connectivity (Figure 7.1.2) with age differed significantly between the patients and controls, whereas no significant interaction was observed in the other frequency bands or power (see the figure legends for details). Please note that the data density is skewed and sparse at the upper end of the age distribution, clearly compromising the comparability of the slopes in the study groups.

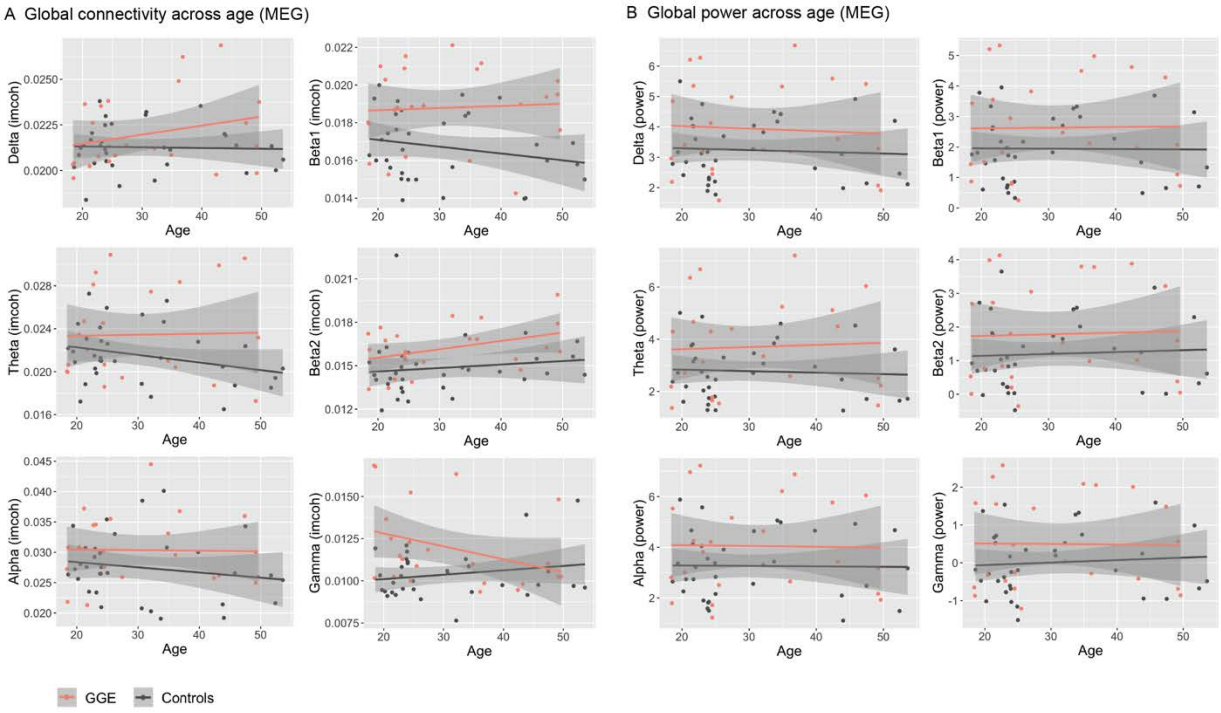

Figure 7.1 | Group-specific trajectories of MEG metrics with age

The plots show individual global (A) connectivity (imcoh) and (B) power across the adulthood for patients with GGE (in red, n = 23) and controls (in black, n = 35) and for the six frequency bands studied. Solid lines represent the fitted linear regression lines for each group, with the 95% confidence interval (shaded area). There was a significant interaction effect of group and age on global connectivity in the gamma frequency band (t_{54} = 1.96, p = 0.03). This effect was also significant in a vertex-based analysis in right temporal regions (data not shown). There were no significant interaction effects in the other frequencies or in power estimates, either for the global markers or at the vertex level. The significance level was set at $p < 0.05$, family-wise error corrected at the cluster level in the vertex-analysis. We estimated linear models to test whether the trajectories of imaging metrics across age differed between patients with GGE and controls in each frequency band using permutation-based analysis (Winkler et al., 2014). The inclusion of covariates in the models, such as sex or MRI scanner, did not change the results. The median age in patients was 26 years (interquartile range 22-40) and in controls 25 years (interquartile range 22-35). Power data was log10-transformed for visualization purposes.

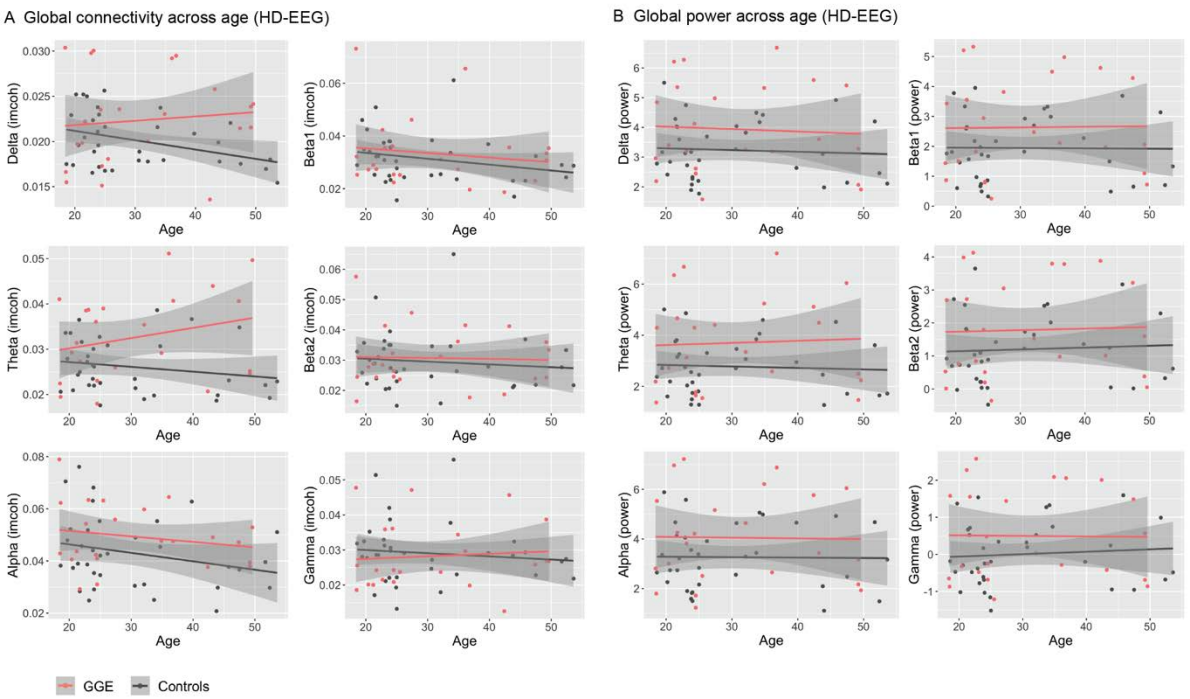

Figure 7.2. | Group-specific trajectories of HD-EEG metrics with age

The plots show individual global (A) connectivity and (B) power across the adulthood for patients with GGE (in red, *n* = 23) and controls (in black, *n* = 35) and for the six frequency bands studied. Solid lines represent the fitted linear regression lines for each group, with the 95% confidence interval (shaded area). There was a significant interaction effect of group and age on global connectivity in the theta frequency band (t_{54} = 1.76, p = 0.04). This effect was also significant in a vertex-based analysis in the insula and right pars opercularis and triangularis (data not shown). There were no significant interaction effects in the other frequencies or in power estimates, either for the global markers or at the vertex level. The significance level was set at $p < 0.05$, family-wise error corrected at the cluster level in the vertex-analysis. We estimated linear models to test whether the trajectories of imaging metrics across age differed between patients with GGE and controls in each frequency band using permutation-based analysis (Winkler et al., 2014). The inclusion of covariates in the models, such as sex or MRI scanner, did not change the results. The median age in patients was 26 years (interquartile range 22-40) and in controls 25 years (interquartile range 22-35). Power data was log10-transformed for visualization purposes.

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List of Abbreviations

AED	Antiepileptic Drug
CAE	Childhood Absence Epilepsy
Cam-CAN	Cambridge Centre for Ageing and Neuroscience
CNV	Copy Number Variations
DMN	Default Mode Network
DZ	Dizygotic
EEG	Electroencephalography
FMRI	Functional Magnetic Resonance Imaging
FWE	Family-Wise Error
GABA	Gamma Amino Butyric Acid
GGE	Genetic Generalized Epilepsy
GGE-GTCS	GGE With Isolated Generalized Tonic-Clonic Seizures
GSWD	Generalized Spike-Wave Discharges
GWAS	Genome-Wide Association Study
HD-EEG	High-Density Electroencephalography
ICA	Independent Component Analysis
ILAE	International League Against Epilepsy
ImCoh	Imaginary Part of Coherency
JAE	Juvenile Absence Epilepsy
JME	Juvenile Myoclonic Epilepsy
MEG	Magnetoencephalography
MZ	Monozygotic
MRI	Magnetic Resonance Imaging
NPC	Non-Parametric Combination
PALM	Permutation Analysis of Linear Models
PET	Positron Emission Tomography
SNR	Signal-To-Noise-Ratio
SUMA	Surface-Based Mapping
TFCE	Threshold-Free Cluster Enhancement
WHO	World Health Organization

DECLARATION

I hereby declare that I prepared the dissertation entitled *"Imaging Network Alterations in Patients With Genetic Generalized Epilepsy and Their Healthy Siblings Using Magneto- and Electroencephalography"* on my own without any sources or aids other than those indicated in the body of this work, references, or acknowledgements.

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Christina Stier
Göttingen, January 2022