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Effects of epilepsy on memory - therapeutic implications, biomarkers, and comorbidities

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Effects of epilepsy on memory - therapeutic implications, biomarkers, and comorbidities

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Editorial: Effects of epilepsy on memory—Therapeutic implications, biomarkers, and comorbidities

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Editorial on the Research Topic
Effects of epilepsy on memory—Therapeutic implications, biomarkers, and comorbidities

Epilepsies are well known to affect memory functions (1, 2), depending on multiple factors, such as type and frequency of seizures (3), the site of the epileptic lesion, the spread of epileptic activity over functional brain regions (4), and possibly processes of inflammation (Li H. et al.).

The best investigated memory disturbance in epilepsy is the common complaint about verbal memory problems among patients with temporal lobe epilepsy [TLE; (5)], especially regarding remote memory and its relation to hippocampal dysfunction (Rastogi et al.). Although reports on memory deficits in epilepsy are numerous, the relationship between memory subdomains and the mediating factors for the negative impact of epilepsy on memory is not clear, yet. For instance, it is less well-known that this deficit is not limited to the semantic domain of memory but interacts with working memory. In this Research Topic, Bolocan et al. demonstrated that patients with drug resistant TLE show impaired working memory, and this deficit contributes to learning deficits and verbal memory problems. Furthermore, the interaction between the number of antiepileptic drugs and the performance in verbal learning and story learning is moderated by working memory (Bolocan et al.).

Disturbance of acquiring verbal memory contents in TLE is also a potential consequence of treating TLE patients *via* surgical resection of the seizure generating tissue. Sala-Padro et al. could demonstrate that there might be additional means to predict the extent of memory decline after surgery. The authors focused on pre-operative resting-state connectivity in functional Magnetic Resonance Imaging (fMRI) in relation to verbal learning decline after anterior temporal lobe resection. They found in both, the pathological and non-pathological anterior connectivity pattern a difference between patients who would experience learning decline and those who remained unaffected. These findings suggest

using pre-operative connectivity patterns as a predictive biomarker for surgical effects on verbal memory in TLE. Indeed, network-level approaches become more and more popular in epilepsy research. It was suggested that also cognitive impairment in epilepsy should be tackled beyond seizures, i.e., from a system-level perspective based on network analysis (Khalife et al.). One additional aspect has to be kept in mind when a patient complains about memory deficits after surgical intervention. In addition to objective factors that determine performance, the patient's subjective perception of memory functioning can differ from objective measures, which is an effect that interacts with depressive symptoms and quality of life (Mücke et al.). In general, depressive mood, and its bi-directional relationship with epilepsies (6), hampers cognitive functions and especially memory—depression screenings are of utmost importance in the neuropsychological assessment of patients with epilepsy.

Beyond TLE, also other forms of epilepsy such as juvenile myoclonic epilepsy (JME) show decreased verbal intelligence, verbal fluency and reading speed (Rainer et al.). These deficits, even when tested with emotional words, are however very subtle and might not be evident in assessments of brain activity (Rainer et al.), emphasizing the need for accurate neuropsychological assessment.

Furthermore, epilepsy-related sleep disturbances were shown to negatively affect memory (7), for example in children suffering from benign epilepsy with centrotemporal spikes a higher percentage of spike and slow wave duration during non-REM sleep is related to impaired cognitive functions and abnormal deactivation in the medial frontal cortex and the posterior cingulate cortex (Li Y. et al.). High frequency oscillations (HFOs, 80–500 Hz) represent another important biomarker that is related to memory consolidation during sleep, but in patients with epilepsy they appear to be rather related to pathology and can be linked to areas with poor memory function (Bruder et al.). In general, age should be taken into account when assessing HFOs as they might interact with aging and aging-associated pathology (Windhager et al.). This is a topic that needs to be investigated better in the future, especially regarding the increased vulnerability of patients with Alzheimer's disease to experiencing seizures (Windhager et al.). A third biomarker of sleep-related abnormalities in epilepsy that was investigated in this Research Topic are interictal epileptiform discharges (IED) that are coupled with sleep spindles. Okadome et al. found that these IED-coupled spindles correlated with lower sleep-dependent consolidation of procedural memory, as assessed in a fingertapping task.

It is well known that medication affects cognitive function and especially memory in patients with epilepsy (8, 9). Medication can have a positive effect not only on seizures but also on psychiatric symptoms and on cognition, but effects on memory have yet to be shown for innovative treatments such as probiotics (Wang et al.).

This Research Topic contributed to an increased awareness for memory deficits in epilepsies and that the assessment of epilepsy effects on memory and cognition shall not be limited to the verbal domain. It highlighted electrophysiological biomarkers in a classical sense (e.g., spikes) as well as modern network approaches. We know today that epilepsies can affect various memory sub-domains, such as verbal memory, remote memory, working memory, procedural memory, but also emotional responses that are linked to memory subdomains. Therapeutic implications of medication and surgical interventions should be investigated in future research in order to provide a better understanding for the origin and nature of memory problems in the individual patient.

Author contributions

YH drafted the editorial and was commented upon and approved by the co-editors/co-authors. All authors contributed to the article and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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BRIEF RESEARCH REPORT
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Working Memory and Language Contribution to Verbal Learning and Memory in Drug-Resistant Unilateral Focal Temporal Lobe Epilepsy

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We aimed to investigate the working memory (WM) and language separate contributions to verbal learning and memory in patients with unilateral drug-resistant temporal lobe epilepsy (drTLE); additionally, we explored the mediating role of WM on the relationship between the number of antiepileptic drugs (AEDs) and short-term verbal memory. We retrospectively enrolled 70 patients with left (LTLE; $n = 44$) and right (RTLE; $n = 26$) drTLE. About 40 similar (age and education) healthy controls were used to determine impairments of groups at WM, language (naming and verbal fluency), and verbal learning and memory (five trials list-learning, story memory—immediate recall). To disentangle the effect of learning from the short-term memory, we separately analyzed performances at the first trial, last trial, and delayed-recall list-learning measures, in addition to the total learning capacity (the sum of the five trials). Correlation and regression analyses were used to assess the contribution of potential predictors while controlling for main clinical and demographic variables, and ascertain the mediating role of WM. All patients were impaired at WM and story memory, whereas only LTLE showed language and verbal learning deficits. In RTLE, language was the unique predictor for the most verbal learning performances, whereas WM predicted the results at story memory. In LTLE, WM was the sole predictor for short-term verbal learning (list-learning capacity; trial 1) and mediated the interaction between AED number and the performance at these measures, whereas language predicted the delayed-recall. Finally, WM confounded the performance at short-term memory in both groups, although at different measures. WM is impaired in drTLE and contributes to verbal memory and learning deficits in addition to language, mediating the relationship between AED number and short-term verbal memory in LTLE. Clinicians should consider this overlap when interpreting poor performance at verbal learning and memory in drTLE.

Keywords: drug-resistant temporal lobe epilepsy, working memory, verbal memory and learning, language, picture naming

INTRODUCTION

Patients with temporal lobe epilepsy show heterogeneous cognitive phenotypes, including various deficits not only in verbal memory and language (1, 2) but also in working memory (WM) (3). WM, a dedicated system for short-term active manipulation of the information, serves as a foundation for various cognitive processes, including verbal learning, language comprehension, and production (4, 5).

Although WM and the short-term verbal memory span concepts significantly overlap (6), they represent distinct entities (7), functionally correlated within a multi-store memory model (8). Short-term memory span refers to the retention of a limited number of data (in the verbal domain, roughly the George Miller's "magical" number 7 ± 2 items) for a relatively short time (usually up to 30 s) (9). Instead, WM involves an active organization and manipulation of the stored information and relates to executive functioning (4).

Findings regarding the WM impairment in drug-resistant temporal lobe epilepsy (drTLE) are discrepant (10–13), most probably due to the different complexity of the tasks used to measure WM. The WM capacity is commonly measured with complex supra-span tasks involving both storage and processing components (14), and it highly depends on the item semantic grouping and complexity (15). Thus, verbal memory tests used in the drTLE assessment, like list-learning and story-learning (16), are differently influenced by WM, having different demands on the semantic processing (17). Although the verbal memory and WM were found to have a high degree of overlap for both tasks (18), other findings suggest that an executive dysfunction impacts the list-learning task performance more than the story memory task (19). In particular, the unrelated list-learning task puts a heavy accent on the rote learning and is considered to be critically dependent on WM (20).

At the same time, language is a powerful predictor for verbal learning and memory (21, 22) and interferes with the verbal memory performance in language-dominant drTLE (23, 24). Therefore, language abilities must be considered when interpreting the verbal memory performance (17, 25), particularly in patients with word-finding difficulties (26).

The poor cognitive outcome is generally associated with an early onset and a long duration of the disease, and with poor seizure control (27, 28). In particular, seizure-related variables, like age at onset, disease duration, and seizure frequency, seem to be more critical than sociodemographic variables, like age and intelligence quotient (IQ), when interpreting results of neuropsychological tests (29). Nevertheless, polypharmacy induces attentional or arousal deficits (30), affecting executive functioning and broader neuropsychological functions (31–33), contributing to cumulating deficits in learning and memory. Executive function and memory showed higher negative correlations with the number of AEDs than with the defined daily dose; therefore, the AED number is a sensitive measure for side effects (34). Executive functions are particularly sensitive to drug load, whereas learning and memory are less vulnerable (35, 36); therefore, the relationship between polytherapy and verbal memory is most likely mediated by executive functions, including the WM capacity (33).

This study aims to understand the unique contributions of the verbal/auditory WM and language in various stages of the verbal learning and memory (i.e., short-term, delayed) of patients with left (LTLE) or right (RTLE) foci, beyond the influence of the primary demographic and disease characteristics. Additionally, we explored the mediating effect of WM on the relationship between the AED number and the short-term verbal memory. We expect the performance at the last list-learning trial and delayed-recall measures to be predicted mainly by language. An opposite pattern of predictors (WM as the main predictor) is expected for the first list-learning trial and story memory (immediate recall), both measures of the short-term verbal memory. We emphasize the importance of the integrated interpretation of the neuropsychological tests used in the pre-surgical assessment of patients with drTLE to unravel the reasons behind the poor verbal memory performance of a patient, thus improving the postoperative cognitive decline prediction and informing the rehabilitation programs.

MATERIALS AND METHODS

Participants and Procedure

We retrospectively enrolled 70 consecutive patients with unilateral drTLE, further divided according to the seizure-onset lateralization into LTLE ($n = 44$) and RTLE ($n = 26$). Patients (right-handed adults, 55.71% male, age: M = 31.77, SD = 9.65 years; age at seizure onset: M = 20.66, SD = 11.69 years; disease duration: M = 10.80, SD = 9.43 years; IQ > 80) were candidates for epilepsy surgery and have been referred between 2019 and 2021 for neuropsychological evaluation by the epileptologists from the Epilepsy Monitoring Unit of the University Emergency Hospital Bucharest. The medical records of patients concluded the diagnosis of unilateral focal drTLE based on the following factors: case history, seizure semiology, interictal and/or ictal video-EEG, and brain imaging (1.5- or 3-T MRI, interictal [18F] fluorodeoxyglucose–PET). Exclusion criteria: moderate or severe mood disorder (e.g., anxiety, depression, and stress); bilateral TLE features; incomplete data. About 40 healthy right-handed individuals (50% female; age: M = 33.90, SD = 9.98) were recruited from the broader community to provide an age and education equivalent control sample to the patient sample. Exclusion criteria for the controls comprised any history of neurologic disease or major psychiatric illness, current psychoactive medication, and IQ < 80. Controls signed a consent form detailing the study procedure under the guidelines from the local ethical committee.

The sociodemographic, clinical, and neuropsychological characteristics of participants are summarized in **Table 1**. There are no significant differences between groups in sex, $\chi^2(2) = 0.88$, $p = 0.645$, $\phi = 0.09$, age at assessment, $\chi^2(2) = 3.09$, $p = 0.213$, $\phi = 0.21$, education years, $\chi^2(2) = 1.63$, $p = 0.443$, $\phi = 0.12$, and IQ level, $\chi^2(2) = 1.13$, $p = 0.568$, $\phi = 0.10$.

Patient groups show similar disease characteristics for epilepsy duration, $U = 572$, $z = 0.00$, $p = 1.000$, frequency of seizures

TABLE 1 | Sociodemographic, clinical, and neuropsychological characteristics of participants.

	LTLE $n = 44$	RTLE $n = 26$	Controls $n = 40$	*p*-value
Age				
y, mean ± SD	33.02 ± 9.92	29.65 ± 8.96	33.90 ± 9.98	0.209*
(range)	(18–57)	(18–49)	(18–56)	
Gender				
F, *n*, %	18 (41)	13 (50)	20 (50)	0.645*
Education				
y, mean ± SD	13.80 ± 2.29	13.65 ± 2.23	14.20 ± 2.28	0.546*
(range)	(11–19)	(11–18)	(11–19)	
IQ				
Inferior (80–89), *n*, %	10 (23)	7 (27)	10 (25)	0.568*
Average (90–110), *n*, %	21 (48)	15 (58)	19 (48)	
Superior (> 110), *n*, %	13 (30)	4 (15)	11 (28)	
Age at seizure onset			-	
y, mean ± SD	21.89 ± 11.39	17.35 ± 11.45		0.069*
(range)	(1–53)	(1–44)		
Duration of epilepsy			-	
y, mean ± SD	10.75 ± 8.70	11.54 ± 10.67		1.000*
(range)	(1–39)	(2–46)		
Seizure frequency / year			-	
mean ± SD	44.27 ± 99.29	133.73 ± 319.37		0.286*
(range)	(1–600)	(2–1500)		
HFC			-	
n, %	2 (5)	4 (15)		0.118*
History of TBI				
n, %	5 (11)	4 (15)	-	0.718*
History of FBTCS				
n, (%)	17 (39)	15 (58)	-	0.122*
No of AED				0.005*
mean ± SD	1.98 ± 0.73	2.54 ± 0.81	-	
(range)	(1–3)	(1–4)		
List-learning capacity				
mean ± SD	48.84 ± 8.96	50.62 ± 9.20	54.95 ± 5.86	0.001*
SE_M	1.35	1.80	0.93	
List-learning trial 1				
mean ± SD	6.20 ± 1.75	6.62 ± 1.47	7.35 ± 1.03	0.004*
SE_M	0.26	0.29	0.16	
List-learning trial 5				
mean ± SD	11.52 ± 2.44	12.42 ± 1.79	13.45 ± 0.93	<0.001*
SE_M	0.37	0.35	0.15	
List-learning delayed-recall				
mean ± SD	9.20 ± 3.02	10.38 ± 3.03	11.88 ± 1.49	<0.001*
SE_M	0.45	0.60	0.15	
Story memory				
mean ± SD	9.95 ± 4.05	11.31 ± 4.63	14.88 ± 2.74	<0.001*
SE_M	0.61	0.91	0.43	
WM				
mean ± SD	9.48 ± 3.50	8.87 ± 3.46	12.07 ± 3.08	<0.001*
SE_M	0.53	0.68	0.49	
Picture naming				
mean ± SD	28.91 ± 2.02	29.96 ± 1.46	30.57 ± 0.64	<0.001*
SE_M	0.30	0.29	0.10	
Semantic fluency				
mean ± SD	19.43 ± 4.94	20.81 ± 5.80	23.52 ± 3.69	0.001**
SE_M	0.74	1.14	0.58	
Phonological fluency				
mean ± SD	16.05 ± 4.00	16.27 ± 4.57	17.80 ± 3.46	0.106**
SE_M	0.60	0.90	0.55	

*IQ, intelligence quotient; LTLE, left drug-resistant temporal lobe epilepsy; RTLE, right drug-resistant temporal lobe epilepsy; HC, healthy controls; HFC, history of febrile convulsions; TBI, traumatic brain injury; FBTCS, focal to bilateral tonic-clonic seizures; AED, antiepileptic drugs; *nonparametric tests (Chi-square test, Kruskal–Wallis test, or Mann–Whitney U-test); **ANOVA with post-hoc Bonferroni corrections, alpha value of 0.05.*

per year, $U = 484.5$, $z = -1.07$, $p = 0.286$, history of febrile convulsions, $\chi^2(1) = 2.45$, $p = 0.118$, $\phi = 0.19$, history of secondary generalized seizures, $\chi^2(1) = 2.39$, $p = 0.122$, $\phi = 0.18$, history of encephalopathy, $\chi^2(1) = 0.24$, $p = 0.627$, $\phi = 0.06$, and age at onset, $U = 721.5$, $z = -1.82$, $p = 0.069$, except higher number of AED in RTLE, $U = 357.5$, $z = -2.78$, $p = 0.005$.

Measures

Verbal memory refers to storing verbally presented information, and tasks include learning word lists (i.e., list-learning) and short stories. The information can be recalled immediately after stimuli presentation or, usually, after 30 min (i.e., delayed recall) (37). Verbal memory was assessed with two tasks: five-trial unrelated list-learning and story memory (immediate recall). The unrelated list-learning test used in our study is an unpublished Romanian adaptation from Ray Auditory Verbal Learning test (38). The subject hears a list of 15 concrete, semantically unrelated words five times and, after each repetition, the subject is asked to immediately recall as many words as possible within 1 min, and freely recall the list after 30 min delay since the last repetition. To disentangle the effect of the short-term memory from learning, we analyzed the performance of patients in three stages of the list-learning test: trial 1—a measure of short-term verbal memory span; trial 5—a measure of learning ability; and delayed-recall, a measure of the retrieval capacity (39), and for the total number of correctly recalled items over the fifth trials (maximum 75), an indicator of the overall verbal learning capacity. The story memory test was an item of the Romanian Mini-Mental State Examination (MMSE)-−2 Expanded Version (40), where subjects must immediately repeat as accurately as possible a short story that is read to them (one trial). The number of correctly rendered keywords (maximum 25) is considered a measure of short-term semantic memory.

For the assessment of verbal/auditory WM, we selected an adapted version (41) of the Letter-Number Sequencing (LNS), a subtest of the fourth edition of the Wechsler Adult Intelligence Scale (42). Subjects listen to an auditory presentation of several series of mixed letters and numbers, increasing in length, and

then reorder the numbers in ascending order and letters in alphabetical order, in increasing difficulty. LNS is a reliable complex span measure for WM used in both clinical and research settings (14, 43, 44).

Naming (or confrontation naming) is defined as the ability to label visually presented stimuli; the picture naming task requires the retrieval of the phonological and semantic information from the memory system (45). Naming abilities were assessed with the Neuropsychological Assessment Battery (NAB) Naming Test (46). The participants need to name the objects in a series of 31 colored pictures.

Verbal fluency is a cognitive function that facilitates information retrieval from memory, measured by asking subjects to retrieve specific information from a specific category or starting with a specific letter, usually within the 1-min time limit (47). Verbal fluency (phonemic and semantic) was assessed by asking participants to say as many words as possible within 1 min, starting with the letter "c," respectively from the "animals" category. The letter "c" was chosen based on the analysis of the frequency of words in the Romanian dictionary as proven to reflect a high-frequency category of words.

Intelligence quotient was assessed with the RAVEN Progressive Matrices Standard test (48), a reliable measure of fluid intelligence. Raw scores were categorized as inferior (corresponding to an IQ range between 80 and 89), average (IQ range between 90 and 110), and superior (IQ > 110). The mood was assessed with Depression, Anxiety, Stress Scale (49), the 21 self-report items version, with the following cutoff scores: 13 for depression, 9 for anxiety, and 17 for stress.

Data Analysis

Demographic characteristics were assessed using one-way univariate ANOVA with the groups (LTLE, RTLE, and controls) as between-subject factors. Patient groups were compared with *t*-tests or Mann–Whitney *U*-test on continuous clinical variables and chi-square on categorical variables.

We computed analyses in three steps. In the first step, we compared participant groups using ANOVA with *post-hoc* comparisons (Bonferroni corrections), based on an alpha of 0.05, supplemented with the Kruskal–Wallis test. The Spearman correlation analysis (alpha value 0.05) was used to explore associations between each criterion variables (verbal memory measures) and predictor variables (sociodemographic and disease characteristics: WM and language), and between predictor variables in each patient group. Kendall or point biserial correlation analyses were conducted between scale and ordinal, and between scale and categorical variables.

In the second step, hierarchical linear regressions were conducted in each patient group, with the verbal memory and learning variables as the dependent variable. The stepwise method was used, and standardized beta (β) values were reported. The hierarchical regression analysis results consist of model comparisons and a model interpretation based on an alpha of 0.05. Each step in the hierarchical regression was compared to the previous step using *F*-tests. The coefficients of the model in the final step were interpreted. For step 1, the relevant sociodemographic and disease characteristics were entered as predictor variables into the null model. Only sociodemographic and disease characteristics variables showing significant correlations with study variable ($r > 0.30$, $p < 0.05$) were first introduced in the regression models. Furthermore, depending on the specific research question for each criterion variable, either WM or language variables were separately added into the model in step 2 and step 3, respectively. At each step, variance inflation factors (VIFs) were calculated to detect the presence of multicollinearity between predictors. Then, variables generating the highest VIF values were subsequently excluded until all VIF values were <2. The following set of sociodemographic and disease-characteristic variables emerged as the most robust predictors in patient groups: education (years), age (at assessment), age at onset, epilepsy duration, frequency of seizures per year, and number of AEDs. However, age at onset was eliminated from almost all regression models as generating very high multicollinearity (VIF > 10). Where only one significant predictor was identified, linear regression was performed.

A Baron and Kenny mediation analysis (50) was conducted in each patient group to assess if WM mediates the relationship between the number of AED and short-term verbal memory measures: list-learning trial 1, learning capacity, and story memory. To determine whether a mediating relationship was supported by the data, three regressions were conducted. For mediation to be supported, four items must be met: (1) the independent variable (AED number) must be related the dependent variable (short-term verbal memory measure), (2) the independent variable (AED number) must be related to the mediator variable (WM), (3) the mediator must be related to the dependent variable but in the presence of the independent variable, and (4) the independent variable should no longer be a significant predictor of the dependent variable in the presence of the mediator variable (41). Only mediations supported by the data were further described in the results section.

Based on findings from the previous two steps, in the third step, ANCOVA was used to compare again the performance of groups at verbal memory while controlling for the relevant predictors.

Effect sizes were reported based on Cohen's standards (51), where coefficients between 0.10 and 0.29 represent a small effect size, coefficients between 0.30 and 0.49 represent a moderate effect size, and coefficients above 0.50 indicate a large effect size. There were no missing data. Correlation and regression analyses were performed with Intellectus Statistics™ (52). All other analyses were performed with SPSS Statistics for Windows, Version 23.0 (IBM Corp., Armonk, NY, USA) (53). *Post-hoc* effect size and achieved power for regression analyses were computed using G*Power 3.1.9.7 tool (54).

RESULTS

Verbal Learning and Memory Performance in Patient Groups

Except for phonemic fluency, $F(2, 107) = 2.297$, $p = 0.106$, $\eta p^2 = 0.04$, participant groups performance analysis (**Table 1**) revealed significant differences between groups at all neuropsychological

tests: list-learning capacity, $\chi^2(2) = 13.73$, $p = 0.001$; list-learning trial 1, $\chi^2(2) = 11.02$, $p = 0.004$; list-learning trial 5, $\chi^2(2) = 14.94$, $p < 0.001$; list-learning delayed-recall, $\chi^2(2) = 19.03$, $p < 0.001$; story memory, $\chi^2(2) = 28.34$, $p < 0.001$; picture naming, $\chi^2(2) = 23.64$, $p < 0.001$; semantic fluency, $F(2, 107) = 7.90$, $p < 0.001$, $\eta p^2 = 0.13$; and WM, $\chi^2(2) = 19.12$, $p < 0.001$. Pairwise *post-hoc* comparisons revealed that, compared to controls, both patient groups had impaired WM (LTLE: $p = 0.002$; RTLE: $p < 0.001$), but only the LTLE group was impaired at verbal memory and language measures: learning capacity ($p = 0.001$), trial 1 ($p = 0.003$), trial 5 ($p < 0.001$), delayed-recall ($p < 0.001$), story memory ($p < 0.001$), picture naming ($p < 0.001$), and semantic fluency ($p < 0.001$), whereas the RTLE group showed only a story memory impairment ($p = 0.002$). Compared to the RTLE group, LTLE showed a lower performance only at picture naming test ($p = 0.017$).

Correlations Between Study Variables

Detailed correlations analysis results are included in **Supplementary Material 1** for all participant groups, including 95% CI. In LTLE, WM showed a strong correlation with list-learning trial 1 ($r_s = 0.53$, $p = 0.002$), and moderate correlations with list-learning capacity ($r_s = 0.46$, $p < 0.001$) and trial 5 ($r_s = 0.39$, $p = 0.009$). Language variables showed moderate correlations with all verbal memory measures (r_s between 0.31 and 0.39, $p < 0.05$). In RTLE, WM showed a strong correlation only with story memory ($r_s = 0.66$, $p < 0.001$), whereas language (mainly picture naming) showed moderate and strong correlations with all verbal memory measures (r_s between 0.39 and 0.51, $p < 0.05$). We found no correlation between WM and language measures in either patient groups. Potential predictors showed no correlation with demographic and disease-characteristics in either patient groups, except a strong correlation between verbal fluency measures and education years in the RTLE group (r_s between 0.54 and 0.56, $p < 0.01$), and a moderate correlation between WM and AED number in the LTLE group ($r_s = -0.37$, $p = 0.014$). The AED number showed moderate correlations with all verbal learning and memory measures only in the LTLE group (r_s between -0.33 and -0.41, $p < 0.05$). IQ level (three levels: inferior, average, and superior) showed small-to-moderate correlations with the list-learning measures not only in LTLE (r_k between 0.33 and 0.45, $p < 0.05$) but also having a strong correlation with education years ($r_k = 0.64$, $p < 0.001$). Education had moderate correlations with list-learning capacity and trial 5 only in LTLE, and with story memory in RTLE, whereas age moderately correlated with list-learning delayed-recall in both groups, and with list-learning capacity in LTLE (r_s between 0.34 and 0.48, $p < 0.05$). We found no significant correlations between sex, history of traumatic brain injury (TBI) or focal to bilateral tonic-clonic seizures (FBTCS), and verbal memory variables in any patient group, except a moderate correlation between history of febrile convulsion (HFC) and list-learning capacity, trial 1 and delayed-recall in LTLE (r_{pb} between 0.31 and 0.41, $p < 0.05$).

In healthy control group, both WM and language showed moderate and strong correlations with verbal memory variables (r_s between 0.32 and 0.80, $p < 0.001$). In addition, WM had strong correlations with all language measures (r_s between 0.61 and 0.67, $p < 0.001$). Education had strong positive correlations with all verbal memory measures (r_s between 0.68 and 0.79, $p < 0.001$), whereas age showed moderate correlations only with list-learning measures (r_s between 0.31 and 0.49, $p < 0.05$). The IQ level strongly correlated with all verbal memory measures (r_k between 0.61 and 0.72, $p < 0.001$), and with education ($r_k = 0.77$, $p < 0.001$).

Predictors of the Verbal Learning and Memory in Participant Groups

The results of regression analyses for variables predicting verbal learning and memory in each participant group are described in **Supplementary Material 2**. **Tables 2–4** summarize the model comparisons for variables predicting verbal learning and memory in each participant groups. Each step was compared to the previous model in the hierarchical regression analysis. After controlling for relevant demographic and disease-characteristic variables, we found an opposite pattern of predictors in patient groups. In LTLE, we found WM as the sole predictor for learning capacity, explaining an additional 10% of the variation ($f^2 = 1.23$, achieved power 0.99), and for trial 1 performance (additional 17%; $f^2 = 0.51$, achieved power 0.98). Semantic fluency was the unique predictor for delayed-recall, explaining an additional 9% of the variation ($f^2 = 0.24$, achieved power 0.80). No significant predictors were identified for trial 5 and story memory. In contrast, in RTLE, we found picture naming as the unique predictor for all list-learning measures (except for trial 1), explaining an additional 40% of the learning capacity variation ($f^2 = 0.33$, achieved power 0.81), an additional 25% of the list-learning trial 5 variation ($f^2 = 0.35$, achieved power 0.83), and an additional 23% of delayed-recall variation ($f^2 = 0.60$, achieved power 0.92). WM was the unique predictor for story memory, explaining an additional 38% of the variation ($f^2 = 1.33$, achieved power 0.99). No significant predictor was identified for list-learning trial 1.

We additionally explored the relationships between WM, language, and verbal memory in the healthy control group. Education and age were introduced in the regression analyses in the first step. However, education was removed from all regression models as generating very high multicollinearity (VIF > 10). WM brought significant additional variation in all verbal memory measures: learning capacity (23%), trial 1 (24%), trial 5 (7%), delayed-recall (8%), and story memory (42%). From language measures, phonemic fluency added a significant amount of variation for all verbal memory measures except for trial 1: list-learning capacity (additional 10%), trial 5 (additional 23%), delayed-recall (additional 27%), and story memory (additional 7%). The regression model for list-learning trial 1 indicates that language did not account for a significant amount of additional variation ($p = 0.066$).

Table 5 summarizes the contribution of predictor variables to the verbal learning and memory performance (final model interpretation) in each of the participant groups (patients and healthy controls) after controlling for relevant disease characteristics and/or sociodemographic variables.

TABLE 2 | Model comparisons for variables predicting verbal learning and memory in LTLE.

Variable (model)	Predictors	R^2	df_{mod}	df_{res}	F	p	ΔR^2
List-learning capacity							
Step 1	education, age, epilepsy duration, frequency of seizures, AED number	0.42	4	39	6.96	<0.001	0.42
Step 2	WM added	0.52	1	38	8.13	0.007	0.10
Step 3	picture naming added	0.56	1	37	3.81	0.058	0.04
List-learning T1							
Step 1	epilepsy duration, AED number	0.22	2	41	5.69	0.007	0.22
Step 2	WM added	0.38	1	40	10.87	0.002	0.17
List-learning T1							
Step 1	education, AED number	0.29	2	41	8.19	0.001	0.29
Step 2	picture naming added	0.34	1	40	3.47	0.070	0.06
Step 3	WM added	0.36	1	39	0.97	0.330	0.02
List-learning delayed recall							
Step 1	age, AED number;	0.25	2	41	6.86	0.003	0.25
Step 2	semantic fluency added	0.34	1	40	5.45	0.025	0.09
Story memory							
Step 1	AED number	0.11	1	42	5.43	0.025	0.11
Step 2	semantic fluency added	0.19	1	41	3.74	0.060	0.07

Each step was compared to the previous model in the hierarchical regression analysis. AED, antiepileptic drugs; LTLE, left drug-resistant temporal lobe epilepsy; WM, working memory.

TABLE 3 | Model comparisons for variables predicting verbal learning and memory in RTLE.

Variable (model)	Predictors	R^2	df_{mod}	df_{res}	F	p	ΔR^2
List-learning capacity*							
	picture naming	0.40	1	24	15.97	<0.001	-
List-learning T5*							
	picture naming	0.25	1	24	7.87	0.010	-
List-learning delayed-recall**							
Step 1	age	0.10	1	24	2.60	0.120	0.10
Step 2	picture naming added	0.33	1	23	7.78	0.010	0.23
Story memory**							
Step 1	education, age at onset	0.21	2	23	3.12	0.063	0.21
Step 2	WM added	0.59	1	22	20.47	<0.001	0.38
Step 3	semantic fluency added	0.59	1	21	0.03	0.862	0.00

**Linear regression; **Each step was compared to the previous model in the hierarchical regression analysis; WM, working memory; RTLE, right drug-resistant temporal lobe epilepsy.*

For the last step of our analysis, participant groups were again compared for verbal memory measures, taking relevant predictors as covariates. When controlling for WM, the results of the ANCOVA were not significant for list-learning trial 1, $F(2, 106) = 2.709$, $p = 0.071$, $\eta^2 = 0.05$, and significant for story memory, $F(2, 106) = 10.527$, $p < 0.001$, $\eta^2 = 0.17$; the mean of story memory for controls (M = 13.93, SD = 3.54) was significantly larger than for LTLE (M = 10.37, SD = 3.41), $p < 0.001$, but similar with RTLE ($p > 0.05$). WM confounds the performance at the short-term verbal memory measures: story memory in RTLE, and list-learning trial 1 in LTLE.

When controlling for age at assessment, education, picture naming, and WM, the results were not significant for learning capacity, $F(2, 103) = 2.128$, $p = 0.124$, $\eta^2 = 0.04$. However, when each of the predictors were controlled separately, we found that only picture naming confounds the performance at the list-learning capacity measure, $F(2, 106) = 2.157$, $p = 0.121$, $\eta^2 = 0.04$. When controlling for education years and picture naming, the results were significant for list-learning trial 5, $F(2, 105) = 4.834$, $p = 0.01$, $\eta^2 = 0.08$; the mean of trial 5 for control (M = 13.08, SD = 1.71) was significantly larger than for LTLE (M = 11.86, SD = 1.73), $p = 0.007$. When controlling for age at assessment, picture naming, and semantic fluency, the results of the ANCOVA were significant for list-learning delayed-recall, $F(2, 104) = 4.419$, $p = 0.014$, $\eta^2 = 0.08$; the mean of list-learning delayed-recall for control (M = 11.35, SD = 2.32)

TABLE 4 | Model comparisons for variables predicting verbal learning and memory in healthy controls.

Variable	Predictors	R^2	df_{mod}	df_{res}	F	p	ΔR^2
List-learning capacity							
Step 1	age	0.29	1	38	15.45	<0.001	0.29
Step 2	WM	0.52	1	37	17.87	<0.001	0.23
Step 3	picture naming and phonemic fluency	0.62	2	35	4.48	0.019	0.10
List-learning T1							
Step 1	age	0.13	1	38	5.91	0.020	0.13
Step 2	WM	0.37	1	37	13.81	<0.001	0.24
Step 3	picture naming and phonemic fluency	0.46	2	35	2.94	0.066	0.09
List-learning T5							
Step 1	age	0.15	1	38	6.66	0.014	0.15
Step 2	picture naming and phonemic fluency	0.38	2	36	6.78	0.003	0.23
Step 3	WM	0.46	1	35	4.83	0.035	0.07
List-learning delayed-recall							
Step 1	age	0.21	1	38	10.39	0.003	0.21
Step 2	picture naming and phonemic fluency	0.48	2	36	9.41	<0.001	0.27
Step 3	WM	0.56	1	35	6.17	0.018	0.08
Story memory							
Step 1	age	0.10	1	38	4.29	0.045	0.10
Step 2	WM	0.52	1	37	31.94	<0.001	0.42
Step 3	picture naming and phonemic fluency	0.60	2	35	3.81	0.032	0.09

Each step was compared to the previous model in the hierarchical regression analysis.

TABLE 5 | Predictor variables for verbal learning and memory measures in participant groups, after controlling for disease characteristics and/or sociodemographic relevant variables (final model interpretation).

		Healthy Controls			LTLE				RTLE			
Verbal memory variable	**Predictor variable(s)**	**β value**	***t*-value**	***p*-value**	**Predictor variable(s)**	**β value**	***t*-value**	***p*-value**	**Predictor variable(s)**	**β value**	***t*-value**	***p*-value**
List-learning capacity	WM Phonemic Fluency	0.34 0.38	2.44 2.81	0.020 0.008	WM	0.30	2.42	0.020	Picture naming	0.63	4.00	<0.001
List-learning Trial 1	WM Phonemic Fluency	0.42 0.38	2.54 2.41	0.016 0.021	WM	0.44	3.30	0.002	-	-	-	-
List-learning Trial 5	Phonemic Fluency WM	0.44 0.37	2.78 2.20	0.009 0.035	-	-	-	-	Picture naming	0.50	2.81	0.010
List-learning Delayed-recall	Phonemic Fluency WM	0.44 0.37	3.06 2.48	0.004 0.018	Semantic Fluency	0.31	2.33	0.025	Picture naming	0.48	2.79	0.010
Story memory	WM Phonemic Fluency	0.62 0.37	4.30 2.73	<0.001 0.010	-	-	-	-	WM	0.61	3.97	<0.001

LTLE, left drug-resistant temporal lobe epilepsy; RTLE, right drug-resistant temporal lobe epilepsy; WM, working memory; β-value, standardized beta coefficient.

was significantly larger than for LTLE (M = 9.81, SD = 2.31), $p = 0.014$.

WM as a Mediator Between AED Number and Short-Term Memory Measures

Our data analyses revealed that the mediating relationship was supported only for list-learning capacity and list-learning trial 1 in LTLE. No mediation relationships were identified in RTLE between WM, AED number, and any of the short-term verbal memory measures.

List-Learning Trial 1 (LTLE)

In step 1 of the mediation model, the regression of AED number on list-learning, ignoring the mediator, was significant, $\beta = -0.95$, $t = -2.80$, $p < 0.01$. Step 2 showed that the regression of the AED number on WM was also significant, $\beta = -1.76$, $t = -2.57$, $p < 0.05$. Step 3 showed that the effect of WM on the list-learning, controlling for AED number, was significant, $\beta = 0.22$, $t = 3.16$, $p < 0.01$, whereas in the presence of the mediator, the AED number was not a significant predictor of list-learning, $\beta = -0.56$, $t = -1.71$, $p = 0.09$. Complete mediation is supported.

List-Learning Capacity (LTLE)

In step 1 of the mediation model, the regression of AED number on list-learning capacity, ignoring the mediator, was significant, $\beta = -5.32$, $t = -3.12$, $p < 0.01$. In step 2, the regression of the AED number on WM was also significant, $\beta = -1.76$, $t = -2.57$, $p < 0.05$. Step 3 showed that effect of WM on the list-learning capacity, controlling for AED number, was significant, $\beta = 0.89$, $t = 2.45$, $p < 0.05$, and AED number was still a significant predictor of list-learning capacity, whereas in the presence of the mediator WM ($\beta = -3.75$, $t = -2.16$, $p < 0.05$). Partial mediation is supported.

DISCUSSION

We conducted this study intending to understand the individual contributions of WM and language in the verbal learning and memory performance of patients with unilateral drTLE, beyond the influence of demographic and disease characteristics. Additionally, we explored the mediating role of the WM on the relationship between the number of AED and short-term verbal memory.

Similar to previous findings (10–12), we found patients with drTLE impaired at WM irrespective of their seizure onset lateralization. In line with the current neuropsychological literature, patients with LTLE were impaired at semantic-related language tasks (picture naming and semantic fluency) and at all verbal-learning measures when compared to healthy controls while showing lower naming abilities compared to the patients with RTLE. Compared to controls, both patient groups were impaired at the story memory test, which is similar to previous findings (17, 55–59).

As we hypothesized, in the two groups, the short-term verbal memory measures were predicted by WM, but differently: the total learning capacity and the first trial of the list-learning task in the LTLE group, and the story memory in the RTLE group. The final comparison with healthy controls revealed that WM confounds the performance at these measures in both groups. The last list-learning trial, a measure of learning ability, was predicted exclusively by language in the RTLE group, while showing no relevant predictor in the LTLE group. In RTLE group, picture naming was the unique predictor for most list-learning measures, whereas in the LTLE group, semantic fluency showed a significant but small contribution on the list-learning delayed-recall measure. Finally, after controlling for relevant predictors, patients with LTLE performed worse than controls only at list-learning last trial, delayed-recall, and story memory tests. In addition, in line with previous research (26), we found that naming abilities confounds the performance at the list-learning capacity measure in the LTLE group. Thus, in line with the literature, only patients with LTLE have verbal memory consolidation and retrieval deficits and a semantic-related impairment.

Many neuropsychological studies addressing the verbal memory performance in drTLE did not consider the contribution of other cognitive processes, like WM and language, on the verbal memory performance. We found both WM and language as significant predictors of various verbal memory components, with WM confounding the performance at the short-term memory measures. Our analyses show that the magnitude of the effect of WM on verbal learning seems to decrease over the list-learning trials, showing no effect on the last trial and the delayed-recall measures, but only in the LTLE group. Conversely, in the RTLE group, WM was associated only with story memory but not with any of the list-learning measures, most probably due to the protective and compensatory role played by the better language abilities in this group. In healthy controls, we found that WM contributes, in addition to language, to all verbal memory measures, whereas being the strongest predictor for the short-term memory measures (list-learning trial 1 and story memory—immediate recall). Thus, our findings support the multi-store memory model (60), where various memory components dynamically interact in the learning process, and control processes such as "rehearsal" play an essential role in the transfer of information from the short-term to the long-term memory (61).

In addition, the current study adds to the findings that semantic function, characterized by deficits in semantic information retrieval (naming, semantic verbal fluency) and impaired performance at semantic verbal memory task (i.e., story memory), is altered in language-dominant patients with drTLE (62). The interdependence between verbal memory and language networks within the left temporal lobe is supported by many neuroimaging studies (63).

Some essential observations can be drawn so far. Story memory is impaired in patients with LTLE independent of their WM abilities. In addition, the list-learning last trial can be considered a better verbal learning ability indicator than the overall learning capacity measure (i.e., the sum of the five trials), proving to be impaired in patients with LTLE independent of language and WM.

As previously suggested (33), our findings showed that WM mediates the interaction between the AED number and the short-term verbal learning measures (i.e., the list-learning first trial and the total learning capacity measure), but this effect was seen only in patients with LTLE. AED was shown to have a differential impact on verbal memory (64), particularly in patients with LTLE (65), mainly when administrated in high doses and polytherapy (66–69).

We found that none of the verbal memory measures differentiated LTLE from RTLE even after controlling for relevant predictors, consistent with other studies (17, 22, 55, 58, 70). However, the story memory task used in our study required immediate recall after only one presentation of the material; therefore, it is representative of the short-term verbal memory. The performance at memory for verbal passages in a learning paradigm showed a significant difference between patients with LTLE and

RTLE (71). As such, it would be more informative to understand the contribution of WM and language on various stages of a story memory learning paradigm, including delayed-recall.

We observed that an opposite pattern of contributors emerged in patient groups. Several reasons might explain this discrepancy. First, the lexical-semantic retrieval adequacy (mainly naming abilities) may be the main reason for this discrepancy, playing a protective and compensatory role for patients with RTLE and overcoming their deficient WM.

Second, the Letter-Number Sequencing task used to measure WM in this study might measure the verbal buffer, a domain-specific "slave system" (43), rather than measuring more complex executive functions (72). Strong neuroimaging evidence connects verbal WM with the left hemisphere (73). In particular, the neuroanatomical basis of the phonological loop is mainly in the left hemisphere, reflecting a strong connection with language networks (74). The verbal learning and memory tests used in this study were delivered to participants in the auditory presentation modality, which presumably relies on the phonological loop (75).

Third, most probable, the language tasks used in our study did not capture the complexity of the language–memory relationship involved by the story memory test. Traditionally, language impairment is well recognized in drTLE for verbal fluency (76) and naming abilities (77), whereas other important language expressive functions, like spontaneous speech and discourse abilities, are much less addressed by the literature (78). Although basic language functions are generally considered unaffected in patients with TLE, the narrative discourse seems to be affected (79), particularly concerning discourse and high-level language abilities (80), probably associated with low WM capacity (81). Patients with early-onset TLE show mild discourse production impairments not associated with other language measures, but correlating with WM (82). In healthy subjects, WM emerged as a robust predictor for high-level language abilities (83). Maintaining verbal information in the verbal WM system directly depends on the long-term representations and processes used in language comprehension and production (84, 85). Retelling a story must involve language production processes as it naturally involves the maintenance and ordering of linguistic information during spoken recall (86). LTLE is particularly associated with macro linguistic disturbances affecting discourse production (87) and spontaneous speech (88). Postoperatively, patients with LTLE were found to be impaired at the immediate recall of stories when compared to those with RTLE, although preoperatively they had similar impaired performance, and this result was not related to the WM capacity, being most probably due to the language network disturbance following left anterior temporal lobe resection (89). Similarly, Joo et al. (2005) (90) found correlations between the extent of left temporal lobe surgery and performance at the story memory test. Thus, the adequacy of the language system of patient, both receptive and expressive, critically influences the interictal verbal learning and memory and must be included in interpreting the memory tests results (78).

Clinical Implications

Working memory is impaired in drTLE and predicts verbal learning and memory deficit, in addition to the language. Clinicians should consider this overlap when interpreting poor performance in verbal learning and memory assessment by including a relevant WM task and examining the learning performance over the five trials of a list-learning test. This is particularly important when naming abilities or other language functions are within the normal range. Our findings suggest that the last list-learning trial is more informative for the verbal learning integrity of the language-dominant hemisphere than the traditional learning capacity measure (the sum of the five trials). Instead, the performance at the first trial showed the highest sensitivity to a WM impairment. Additionally, an impairment at the story memory test in the absence of a WM deficit may suggest that the functional integrity of the language-dominant hemisphere is affected. Concurrently, a deficit at the story memory test should be interpreted with caution when WM is impaired.

Limitations

Our patient group included various pathologies; therefore, we could not disentangle the effect of mesial or lateral temporal lobe epilepsy on study variables. In addition, besides hand-dominance, we did not use another modality to determine the language lateralization; therefore, we can only assume that the majority of our patients have the left hemisphere as language-dominant (91, 92). Although the sample sizes of patients are small, particularly for RTLE group, the *post-hoc* effect sizes and the achieved power for regression analyses showed significant results in each group. In addition to the AED number, further research should explore the mediating role of WM on the relationship between specific drugs (i.e., type, dosage, and combination) and verbal memory performance in drTLE.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The name of the repository and accession number can be found below: Mendeley Data, https://data.mendeley.com, doi: 10.17632/hhwh69wy6n.1.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Board of the University of Bucharest, Romania (notice no. 329/11.12.2019). The participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

MB and CI contributed to the conception, the design of the study, and its implementation. MB wrote the first draft of the

manuscript. CI wrote sections of the manuscript. CI and EA reviewed the manuscript. All authors read and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2021.780086/full#supplementary-material

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Are High Frequency Oscillations in Scalp EEG Related to Age?

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Background: High-frequency oscillations (HFOs) have received much attention in recent years, particularly in the clinical context. In addition to their application as a marker for pathological changes in patients with epilepsy, HFOs have also been brought into context with several physiological mechanisms. Furthermore, recent studies reported a relation between an increase of HFO rate and age in invasive EEG recordings. The present study aimed to investigate whether this relation can be replicated in scalp-EEG.

Methods: We recorded high-density EEG from 11 epilepsy patients at rest as well as during motor performance. Manual detection of HFOs was performed by two independent raters following a standardized protocol. Patients were grouped by age into younger (< 25 years) and older (>50 years) participants.

Results: No significant difference of HFO-rates was found between groups [$U = 10.5$, $p = 0.429$, $r = 0.3$].

Conclusions: Lack of replicability of the age effect of HFOs may be due to the local propagation patterns of age-related HFOs occurring in deep structures. However, limitations such as small sample size, decreased signal-to-noise ratio as compared to invasive recordings, as well as HFO-mimicking artifacts must be considered.

Keywords: high frequency oscillation, electroencephalogram, scalp-EEG, HD-EEG, epilepsy

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INTRODUCTION

In the last 2 decades high frequency oscillations (HFOs) have been studied extensively (1, 2). These HFOs are considered as oscillatory field potentials in the gamma- or high gamma band standing out of the oscillatory background activity showing a regular morphology that can be classified as ripples (80–250 Hz) and fast ripples (>250 Hz) (3). Bragin et al. (4) found that HFOs, in particular fast ripples, are typical for the epileptogenic region and may be a correlate of pathological changes leading to hypersynchronously bursting pyramid cells (5, 6). Therefore, HFOs are considered as a candidate marker for epilepsy (1) not only in adults but also in neonates (7) and children with refractory epilepsy (8).

Besides the assumed importance of HFOs in epilepsy diagnosis, earlier studies have shown that activity in this higher frequency range can also represent a physiological mechanism for synchronization of interneural firing not only of coherent neurons but also of neurons in spatial separated cortical areas (9–11). Further, HFOs recorded in both healthy and unhealthy patients can be seen during various cognitive processes such as memory-consolidation

(12–14) motor-performance (15), and visual perception (16, 17). Thus, HFOs might not be displaying a genuine pathological characteristic in patients with neurological disease. However, the pathological relevance of this new biomarker may come into play when physiological mechanisms are disturbed.

What we know today about HFOs is largely based on empirical studies which investigated under what circumstances the phenomenon can be observed. Using invasive recordings as well as scalp EEG, scientists aimed to gather information about this promising biomarker in neuroscience (2, 18, 19). It is still a major challenge to identify potential indicators for a valid differentiation between pathological and physiological HFOs. By delineating particular characteristics like the background oscillatory activity (10), phase locking of HFOs and low frequencies recorded during sleep (20–22), spatial and signal characteristics (23) such as the amplitude (24), morphology (25), the occurrence in relation to an epileptic spike (26, 27), current research strives to contribute important information concerning the differentiation of physiological and pathological HFOs.

HFOs have been observed most often in invasive EEG recordings, using intracranial implanted macro- and micro-electrodes in patients with refractory focal epilepsy therefore undergoing presurgical examination (28). These studies reported a link between fast ripples and interictal spikes which are a common marker for identification of the seizure onset zone (SOZ). It would be advantageous to be able to examine HFOs in scalp EEG, in order to make this biomarker available also to patient populations without the necessity for an invasive recording. Because of the small amplitude and local propagation characteristics of HFOs it was highly debated whether HFOs can be detected in scalp EEG (16, 29). Scalp-EEG measures the summated activity of large populations of neocortical neurons and is much more prone to exogenous artifacts and noise. Local propagation patterns of HFOs (30) and their small size in relation to prominent muscle and movement artifacts in scalp EEG put the undertaking of detecting HFOs in the scalp into question. Recent work suggests that it is indeed possible (31, 32). It was suggested that a high-density (HD-EEG) would be advantageous over a conventional 10–20 montage; more specifically, by using HD-EEG, corresponding areas could be identified between scalp and invasive recordings (31).

Identification of HFOs in scalp-EEG is not straightforward (33, 34). First, the presumed pathological form of HFOs is likely to co-occur with physiological HFOs in scalp EEG (35) such that methods for the distinction of these phenomena are highly warranted. Second, it is crucial to increase the signal to noise ratio (36, 37), third, to use adequate spatial sampling (31), and, fourth, to reduce and correctly identify non-neural signals, so called artifacts, in order to allow application of scalp-based HFO analysis in clinical routine. Recent developments of custom-made low-noise amplifiers (34, 37) allow for a more accurate identification of HFOs in scalp EEG, which also correspond well with invasively recorded HFOs (34). However, the distinction of pathological from physiological HFOs in scalp EEG is a topic of its own merit, as these two phenomena are highly similar (33). A common approach is to compare HFOs in epileptic and non-epileptic EEG (33), but it is so far impossible to distinguish pathological from physiological HFOs on an individual case basis within a single recording. Nevertheless, because of their specificity scalp-HFOs bear a big potential to serve as interictal markers for epilepsy (38).

Scalp-EEG bears a further challenge, namely age. The EEG changes with age (39), and therefore it is also discussed as a factor that contributes to the development of epileptogenicity, due to a complex interaction between epilepsy and other diseases of the aging brain like stroke, dementia or traumatic brain injuries (40). From a structural view, the pathophysiology of temporal lobe epilepsy does resemble premature brain aging (41). As a consequence, the prevalence of active epilepsy increases with age (42) and might explain the high incidence of temporal lobe epilepsy in the elderly. Further investigations conducted by Tombini et al. (43) showing a link between the extent of neural degeneration resulting from diseases like Alzheimer's disease (AD) and epilepsy may be of interest since the number of HFO may predict the severity of epilepsy, therefore reflecting the extent of neural degeneration. Accordingly, HFO may also be used as a marker for the classification of neurodegenerative disorders such as AD in the future.

If we assume that the increase of HFOs in patients with epilepsy is due to structural changes resembling premature brain aging (41), it must be considered that HFO rate occurs to a greater extent in elderly patients independent of disease. Following this it can be hypothesized that a certain amount of HFO increase in elderly epilepsy patients is due to structural changes rather than simple pathological reasons. This implies that HFOs might not sensitively differentiate between aging effects and epileptogenic processes. Therefore, we claim that there is a need to understand how HFO rates change in elderly patients.

Previous research reported a link between age and HFO occurrence in invasive EEG (44). However, this finding was so far not replicated in scalp EEG. A recently conducted study by Cserpan et al. (45) found, that HFO activity in the ripple band is increased in a pediatric population of epilepsy patients compared to older subjects. They found that younger children showed a higher HFO compared to older children. Studies like these show that it is indispensable to estimate the contribution of age to the likelihood of HFO occurrence.

If older patients are more likely to exhibit HFOs regardless of epileptic pathology, HFOs may not be a reliable marker for epilepsy in this age group. The occurrence of high HFO rates in elderly patients with epilepsy might just be more diffusely related to physiologic than pathophysiologic changes that are a consequence of aging.

Using scalp-EEG we examined a possible relationship between age and HFO activity by comparing HFO rates between older and younger patients suffering from epilepsy as well as healthy controls.

MATERIALS AND METHODS

Recruitment

We prospectively enrolled patients admitted to the Epilepsy Monitoring Unit (EMU) at the Department of Neurology, Christian-Doppler Medical Center Salzburg. Patients were

TABLE 1 | Patient information.

ID	Sex	Age	Hand.	AoO	Epilepsy	Seizure type
< 25 Years						
004	F	24	Right	5	TLE right	Focal aware and focal to bilateral tonic-clonic seizures
022	F	23	Left	20	MTLE right	Focal impaired awareness seizures
023	F	22	Right	3	PTME Left	Focal aware seizures; Focal impaired awareness seizures; Focal to bilateral tonic-clonic seizures
024	M	19	Right	4	OLE Right	Focal aware seizures; Focal impaired awareness seizures; Focal to bilateral tonic-clonic seizures
028	M	19	Right	5	FLE	Focal aware seizures; Focal impaired awareness motor seizures; Focal to bilateral tonic-clonic seizures
029	F	22	Left	20	NFD	Focal to bilateral tonic- clonic seizures
> 50 Years						
002	M	56	Right	42	TLE left	Focal aware seizures
007	F	59	Right	59	TLE left	Focal impaired awareness seizures
037	M	64	Left	63	NFD	Focal to bilateral tonic- clonic seizures
039	F	54	Right	53	NFD	Speech impairment; cognitive restrictions; vertigo
042	M	55	Right	53	NFD	Focal to bilateral tonic- clonic seizures

M, Male; F, Female; AoO, Age of Onset; Hand., Handedness; NFD, no final diagnosis; TLE, Temporal Lobe Epilepsy; MTLE, Mesial Temporal Lobe Epilepsy; PTME, Posttraumatic Multifocal Epilepsy; OLE, Occipital Lobe Epilepsy; FLE, Frontal Lobe Epilepsy.

admitted to the hospital for epilepsy clarification using video-EEG. Recruitment for the study was done one week prior to admission via phone. On the day of admission, patients who agreed to participate got informed about the purpose and possible side effects and signed informed consent prior to starting the experimental procedure.

Ethics

The study was conducted in accordance with the recommendations of good clinical practice and was approved by the local ethics committee (Ethik-Kommission für das Bundesland Salzburg: E/1755, 415-E/1806/4-2014, initial approval on 30/03/2014, latest amendment on 11/07/2016).

Participants

Between February 2018 and December 2020 48 participants had been enrolled to the study. For the analysis at hand only complete datasets of participants younger than 25 and older than 50 years with a sufficient signal to noise ratio (SNR) and minimal artificial distortions were included. We also excluded data from patients with extensive motor symptoms according to diagnosis. EEG segments with sufficient signal quality were exported. According to their age participants were assigned to one of two groups: *< 25 Years* vs. *> 50 Years*. In the *< 25 Years* group we excluded 2 patients because final diagnosis made by the physician did not confirm an epileptic disease (1 male patient with non-epileptic psychosis and 1 female patient suffering migraine). In the *> 50 years* group we excluded 3 patients because final diagnosis made by the physician did not confirm an epileptic disease (2 women with epileptiform discharges of unclear cause and 1 woman with restless-legs syndrome). Excluded patients served as controls. In patients labeled with "no final diagnosis" physicians where not absolutely sure about hemispheric localization of seizure onset zone.

11 Patients met all Criteria ($n_{<25} = 6$; 4 Women; 2 Righthanded; $M_{<25} = 21.17$ Years, $SD_{<25} = 2.14$ Years; $n_{>50} = 5$; 2 Women; 2 Righthanded; $M_{>50} = 57.2$ Years, $SD_{>50} = 3.83$ Years). For a Detailed Overview of Patient Information see **Table 1**.

Regarding the control group, the study at hand used patients retrospectively diagnosed as "non-epileptic" by the physicians at the hosting institution. Since the under-25 age group contained only 2 and the over-50 age group contained only 3 subjects, controls were combined into a common control group (see **Table 2**). This results in a rather small sample ($n_{contr.} = 5$; 4 women; 5 righthanded; $M_{contr.} = 38.6$ years, $SD_{contr.} = 17.9$ years). In future studies this should be considered and larger control groups should be recruited in order to distinguish physiological aging from epilepsy-associated processes and their relation to HFO activity.

Data Acquisition

Recording of high-density (HD) EEG was performed at the first day of hospitalization before starting the video-EEG monitoring. We decided to use HD-EEG, because previous studies reported a higher correspondence of areas with high ripple rates between intracranial EEG and HD-EEG compared to 10–20 EEG (31). A study conducted by Avigdor et al. (46) retrospectively analyzed EEG post-surgical recordings of patients with drug resistant epilepsy and found that the highest rates of HFOs can be found within the resected areas. Using both HD-EEG and the reduced 10–10 as well as the 10–20 EEG they showed that the detection of HFOs is even more accurate when conducted using HD-EEG and can be used for the identification of the epileptogenic zone (EZ.) They stated that an increase of localization accuracy of 40–60 percent can be achieved.

TABLE 2 | Control group.

ID	Sex	Age	Hand.	AoO	No. of HFOs
026	F	20	Right	5	4
027	M	18	Right	20	9
020	F	52	Right	42	0
043	F	52	Right	59	17
046	F	51	Right	63	0

ID, Identification number; M, Male; F, Female; Hand., Handedness; AoO, Age of Onset; No. of HFOs, Number of HFOs.

The experiment was performed using Presentation® software (Version 18.1, Neurobehavioral Systems, Inc., Berkeley, CA). HD-EEG recording was performed using a 256 channel EGI Hydrocel Net (Net Station Acquisition 5.0, Electrical geodesics, Inc.). Prior to the EEG recording, impedances of the electrodes were checked and kept within a range of 50 to 100 kΩ. Electrode number 43 was excluded in all recordings because of a hardware problem.

To cut out powerline noise a 50 Hz notch filter was applied. Sampling rate was 1 kHz, fulfilling Nyquist- criteria and a high pass filter of 0.1 Hz enabled elimination of low frequencies outside the spectrum of interest. In order to guarantee high data quality, patients were asked to intentionally generate artifacts during an initial calibration recording, which served as a reference for postprocessing artifact exclusion. Participants were asked to blink, raise their eyebrows, swallow and chatter their teeth several times in succession. To control for facial muscle activity, an electromyogram (EMG) was recorded from the patients' cheek. Participants were encouraged to move as few as possible between the test segments in order to minimize artificial distortion in the EEG.

Experimental Procedure

Patients were seated in a comfortable chair. During the initial resting recording, patients were asked to sit as relaxed as possible with closed eyes for one minute. Next, a fingertapping task comprising 12 learning and 5 recall trials was conducted. Each of the trials lasted for 30 seconds and was separated by a short intertrial break of equal duration. The task used was the same as described by Gerner et al. (29). Participants were instructed to repeatedly type a five-digit sequence presented on the screen as fast and accurate as possible with their non-dominant hand. The task included a practice-, a learning-, and a recall-part. Following previous studies, showing a link between number of HFOs and neural learning processes in the somatomotoric cortex (5), we decided to analyze the learning part in the study at hand to maximize probability of event detection. During the intertrial breaks, participants were told to relax their fingers on the keyboard to minimize artificial activity. Prior to the experiments recall-phase, a resting phase of ten min was recorded. In total, the testing-procedure lasted for about 1.5 h.

HFO Identification/Marking Procedure

The marking procedure to identify HFOs closely followed the procedure as presented in previously published research (19, 29). Data was analyzed using an in-house built software for HFO analysis called MEEGIPS (33). For the analysis we extracted EEG-segments with a maximal SNR and minimal artificial distortion of 198 seconds duration overall including initial rest, pause rest as well as the breaks between the single fingertapping segments (i-rest + p-rest + breaks of fingertapping). EEG-segments of 60 seconds duration were extracted from the learning-part of the fingertapping task (for composition of segments see **Figure 1**).

Segments were analyzed manually and independently by two experienced raters. HFO identification was blinded with respect to epilepsy- lateralization and diagnosis to minimize rater-bias. In a first step, the raters visually identified the events of interest independently. Due to several factors of manual analysis of scalp EEG in general and HD-EEG in special, interrater reliability was not available. Because of this, we decided to take into account all events marked by the two raters independently. For the final analysis, the detected events were reviewed by both raters until an agreement was reached regarding the classification of the specific events (ripple vs. unclear HFO vs. artifact).

A total of 124 channels per segment were analyzed. Prior to HFO marking, two different sets of filters were applied to the data. A *finite impulse response filter* (FIR) labeled as *multifilter* with a frequency-range of 50–500 Hz was applied. We used this filter to remove the harmonics of line noise from the EEG-data and the FIR provides a higher constancy in phase-delay. The so-filtered data was obtained to enable identification of frequency activity typically seen in non-neural events like motor-dependent muscle movement. Afterwards, the same segment was filtered using an FIR in the gamma band with a frequency range of 80–250 Hz for the purpose of a detailed HFO analysis. Both versions of data (filtered at 50–500 Hz and at 80–250 Hz) were viewed simultaneously (see **Figure 2**).

The gamma-range filtered EEG-segment (80–250 Hz) was viewed with a time resolution of 450 mm/s, a timescale of 0.6 seconds per view and an amplitude scaling of 3,00 μV. Standard scaling with zero mean corrected and 50–500 Hz filtered data was placed on the right side of the desktop. Time resolution was 310 mm/s and amplitude scaling was 1,10 μV. Timescaling was 0.2 second per view.

Raters always analyzed groups of 6 channels simultaneously plus an additional EMG channel. Grouping of channels was based on spatial proximity to each other. Channels with low SNR or continuous artifacts were excluded. However, excluded channels were not blinded but merely marked with a red layer to serve as a reference for artifact identification (See **Figure 2**). According to Wang et al. (47) the ROI was defined over the central (C3, C4), the adjacent frontal (F3, Fz, F4) and parietal (P3, Pz, P4) region corresponding to the 10–20 montage of the scalp EEG. HFOs recorded during motor task performance were previously related to motor planning (48) and motor action (49). Therefore, the ROI should cover the premotor, motor and somatosensory cortices. For HFO analysis in the resting EEG, the ROI should cover a large region including the fronto-parietal default mode network (50). To minimize contamination effects in statistics due to false positives generated by muscle artifacts mimicking HFO's we previously excluded channels spatially related to cranial muscles (e.g. orbicularis oculi muscle, occipitofrontalis muscle, temporal

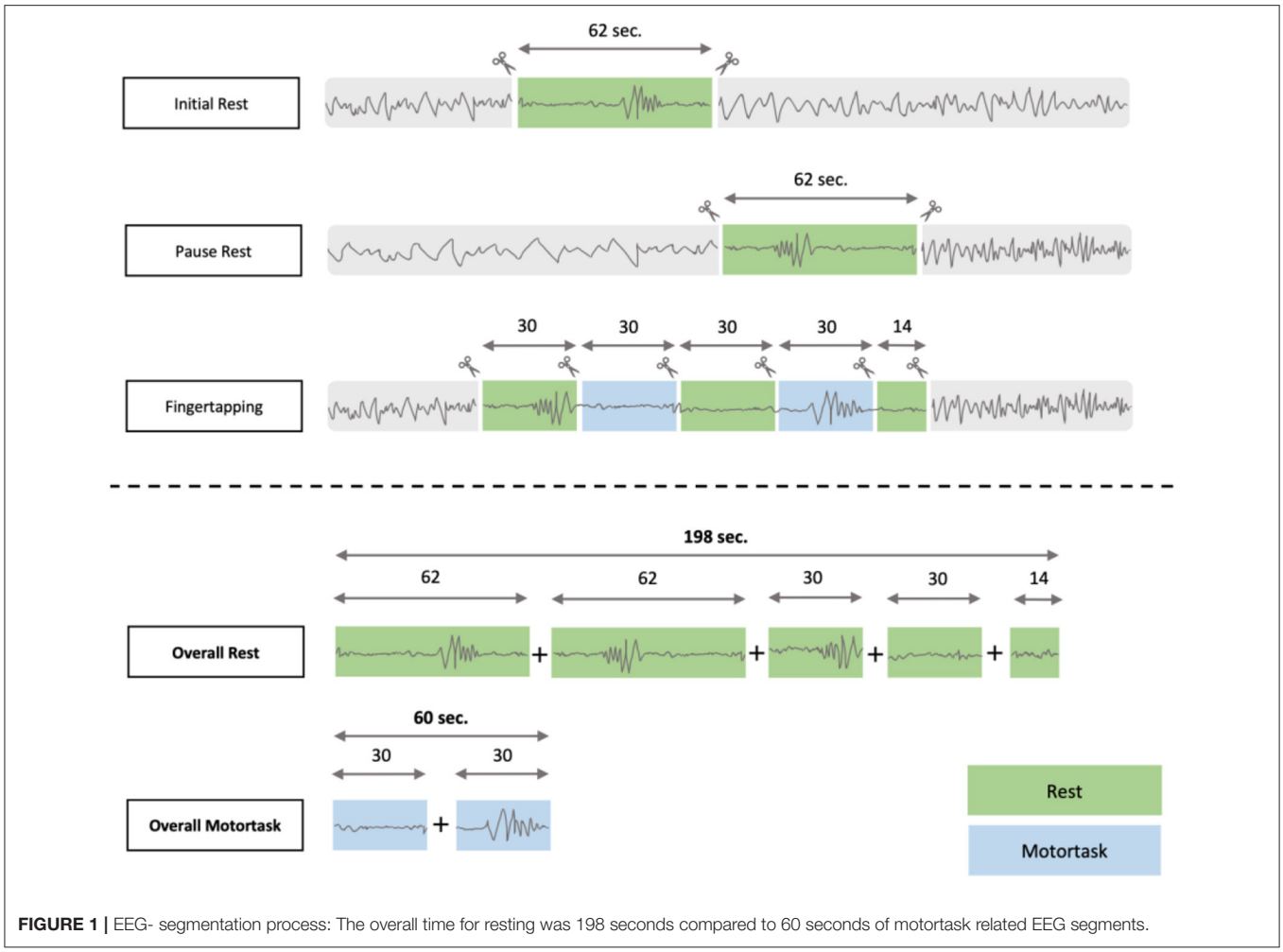

FIGURE 1 | EEG- segmentation process: The overall time for resting was 198 seconds compared to 60 seconds of motortask related EEG segments.

muscle). The 124 channels were the same for all patients and were shared symmetrically between the hemispheres. Channels of interest were chosen relating to their corresponding areas associated with a higher activity during different tasks (48–50). Additionally, we excluded all channels that showed continuous artifacts, no signal at all as well as channels with poor signal to noise ratio. By doing so, we reduced the number of channels from 256 to 124. Events of interest (EoI) that correlated with eye blinks within a range of 200ms before and after the event were also excluded. To do so, we used some of the frontal electrodes of the HD-EEG net as electrooculographic electrodes.

An analysis pane (**Figure 3**) with a set of configurable tools for evaluation of the selected EEG data was enabled for each of the displayed windows (33). The following representations were used for analysis of potential HFOs:

1. Empirical Mode Decomposition (EMD): Highpassfilter based on EMD (51).
2. Differential Fourier Transform (DFT).
3. Discrete Wavelet Packet Decomposition (DWPD): Signal-Power extraction over the segments using discrete time and frequency steps.
4. CWT: Continuous Wavelet Transform.

Events of interest (EoI) were marked by each rater and then classified using the information provided by the analysis pane. EoI's were classified into 3 categories, based on recent research investigating HFO-identification on the scalp EEG (29, 38, 52). Events consisting of at least 4 consecutive oscillations showing a regular morphology were considered as HFOs. The signals should stand out significantly from the background with respect to their amplitude and a superimposed activity should be visible in the multi-filtered raw data. A superimposed activity is an oscillation of high frequency, which "rides" on an oscillation of lower frequency.

Classification of events was performed as follows:

(i) Events showing an isolated high frequency activity in the high pass filtered EEG and a superimposed activity in the multifilter were considered as HFOs (ripple).
(ii) Events showing both, a high frequency activity in the high pass-filtered EEG as well as discrete "blob" in the DWPD however no superimposed activity in the raw data, were designated as unclear HFOs *(uHFO)*.

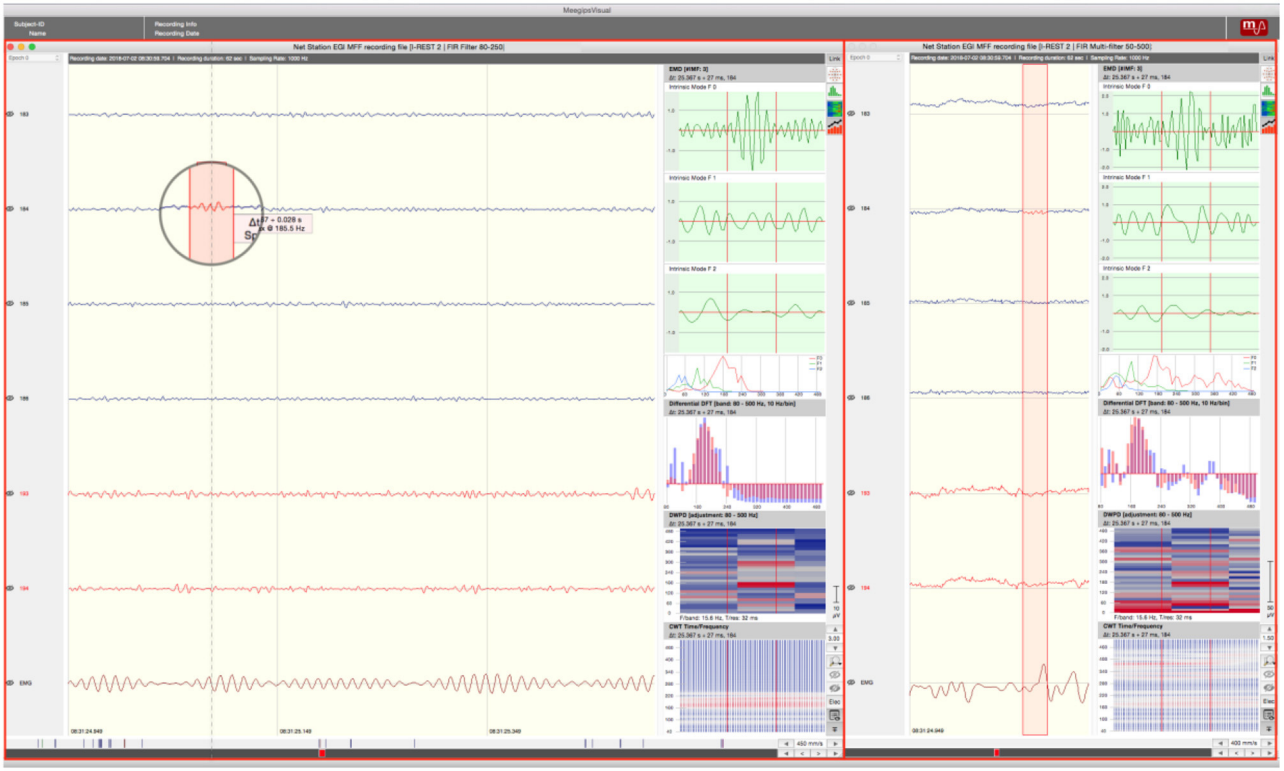

FIGURE 2 | Screen Arrangement MEEGIPS: **Left side window** depicts FIR filtered data (80–250Hz); **Right side window** depicts multifiltered data (50–500Hz). An overlap between marked event (red window in circle) and EMG activity can be seen. Therefore this event would have been marked as an artifact. Red marked channels were excluded.

(iii) Events that did not fulfill the criteria of an isolated blob neither showed a superimposed activity in the raw data were considered as artifacts *(genArt)*. Activity showing a correlation between the observed EEG and the EMG activity were assumed to be triggered by muscle activity and also marked as artifacts.

Table 3 gives an overview about event classifications. An isolated blob shows at which frequency the oscillation has its maximum, i.e. where the signal power is located. Thus, an isolated blob is a quality mark that gives information about the "clarity" of the oscillation. When oscillations of various frequencies overlap the pattern in the DWPD looks more widespread. Artifacts show a broader representation in the frequency spectrum whereas HFO events appear as isolated "islands". Thus, DWPD can be used to exclude artifacts and verify true events.

Statistical Analysis

Statistical analysis aimed to answer the question whether there is a significant difference between the groups of young and elderly patients regarding mean HFO rates. Statistical analyses were carried out using the IBM SPSS Statistics Software (IBM Corp. Released 2011. IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp). Due to the small sample size, we conducted non-parametric tests. Rates of ripples and unclear HFOs identified during resting phases as well as during learning trials of the fingertapping task were calculated for each patient. The resting phases included the events identified during initial rest, pause rest, and the resting trials of the motor learning task. Because of the relatively small number of identified ripples, we also looked at the overall HFO rate, where in addition to ripples, we also included unclear HFOs ($HFO = ripples + uHFO$).

To test for significant group differences between younger and older patients, *Mann-Whitney tests* were carried out, comparing the ripple rates and the overall HFO rates both during the motor learning task and during resting phases. Because of multiple comparisons, all results were interpreted at the Bonferroni corrected level of significance $p = 0.05/6 = 0.008$.

Furthermore, we also conducted a semi-parametric repeated measures ANOVA with the factors age and condition to compare the detected HFOs during rest with those recorded during motor tasks.

We chose this method that only requires metric data but allows for non-normality and variance heterogeneity (53). This method is implemented in the function RM of the R-package MANOVA.RM (54). We used it with the parametric bootstrap with 1000 iterations. The parametric bootstrap showed the most favorable performance in unbalanced designs.

Since the analyzed EEG segments differed between the tasks (resting vs. motor-task) with respect to their length (resting: 198

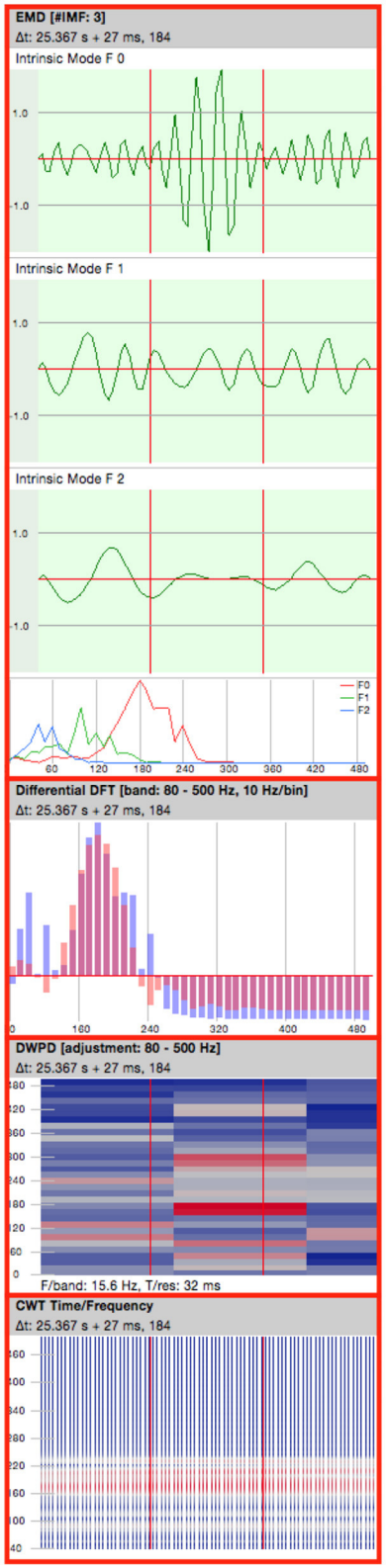

FIGURE 3 | Analysis pane. EMD, Empirical Mode Decomposition (4 consecutive oscillations are shown); DFT, Differential Fourier Transform; DWPD, Discrete Wavelet Packet Decomposition; CWT, Continuous Wavelet Transform.

TABLE 3 | Classification criteria and categories of events: Filtered: HFOs visible in High pass filtered data; Blob: isolated Blob visible in DWPD; Raw Data: superimposed activity visible in multi filtered raw data; Eventtype: High frequency oscillation (HFO); unclear high frequency oscillation (uHFO); generic Artifact (genArt).

Filtered	Blob	Raw Data	Eventtype
✓	✗	✓	*HFO (Ripple)*
✓	✓	✗	*uHFO*
✓	✗	✗	*genArt*

sec.; motor-task: 60 sec.), the HFO rate per minute was calculated for each patient. **Figure 4** provides an overview about mean HFO rate of patients.

A control group was included to test for significant differences between patients suffering from epilepsy and patients with no epilepsy diagnosis. The control group was composed of EMU patients whose clinical picture was determined not to be a form of epilepsy. *Mann-Whitney tests* were carried out, comparing the ripple rates and the overall HFO rates both during the motor learning task and during resting phases. Because of multiple comparisons, all results were interpreted at the Bonferroni corrected level of significance $p = 0.05/6 = 0.008$.

To investigate a possible influence of sleep on mean HFO rate, we calculated a correlation between the two variables. The amount of sleep was based on patients' information about the hours they have slept the night before the testing.

To investigate a possible lateralization effect, we calculated correlations between the patients' age and the number of HFOs detected in the epileptic hemisphere, and in the non-epileptic hemisphere, respectively. Furthermore, we also calculated correlations between the patients' age and the number of HFOs detected during the motor task, and during resting phases, respectively. Because of multiple comparisons, all results were interpreted at the Bonferroni corrected level of significance $p = 0.05/4 = 0.0125$.

RESULTS

Difference of HFO Rate; Young vs. Elderly Patients

The analyses were conducted separately for ripples, and ripples including unclear HFOs. Regarding the events detected while patients rested, a Mann-Whitney test indicated that there was no difference in the number of ripples between the younger and the older group of patients ($U = 10.5$, $p = 0.429$, $r = 0.3$).

Even after including unclear HFOs in the analysis, the Mann-Whitney test indicated that there was no difference between the two patient groups regarding the mean HFO rate during resting ($U = 8.5$, $p = 0.247$, $r = 0.36$).

During the learning trials of the fingertapping task, no clear ripple was identified in any of the patients. Regarding overall HFOs (*ripple + uHFO*), again the Mann-Whitney test indicated that there was no difference between the younger and the older group of patients ($U = 14.0$, $p = 0.931$, $r = 0.06$).

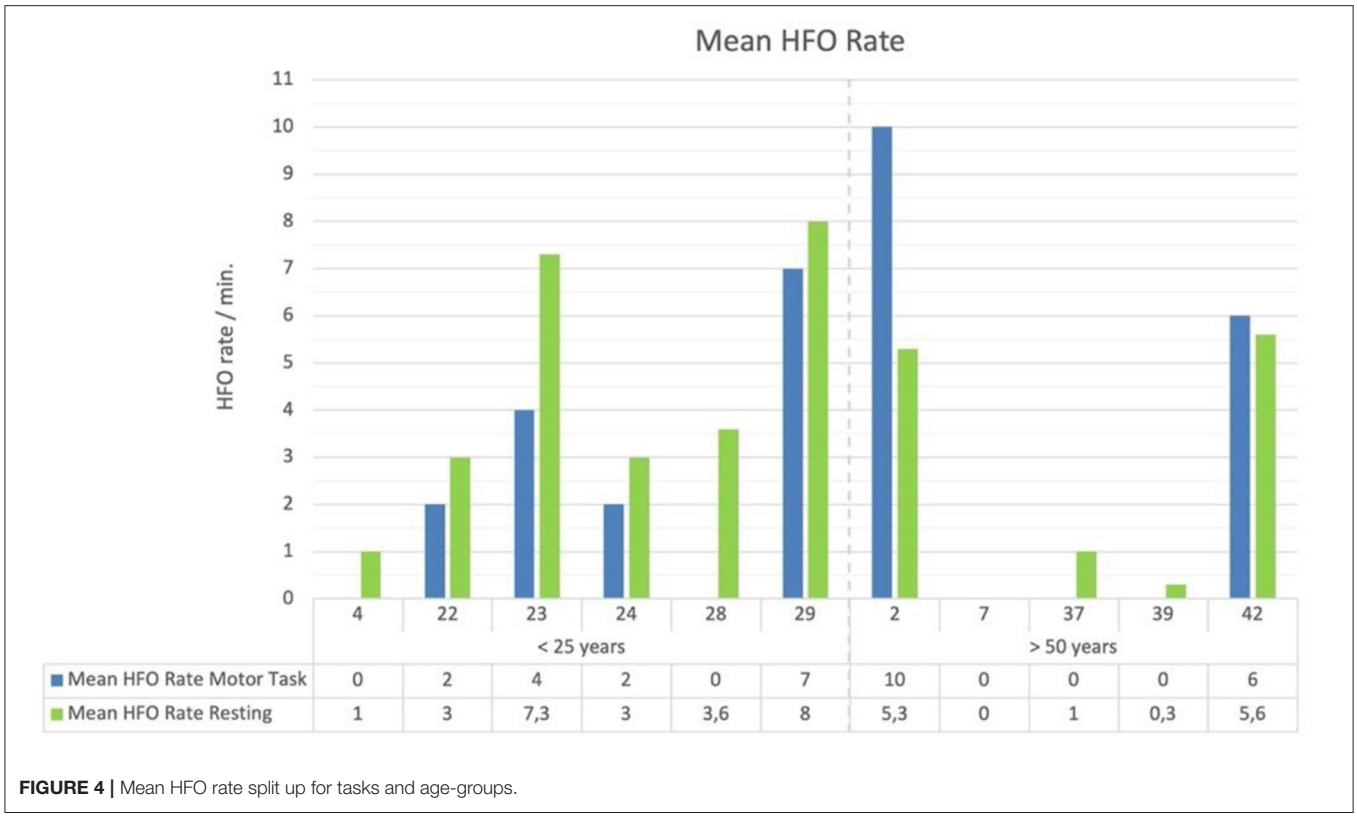

FIGURE 4 | Mean HFO rate split up for tasks and age-groups.

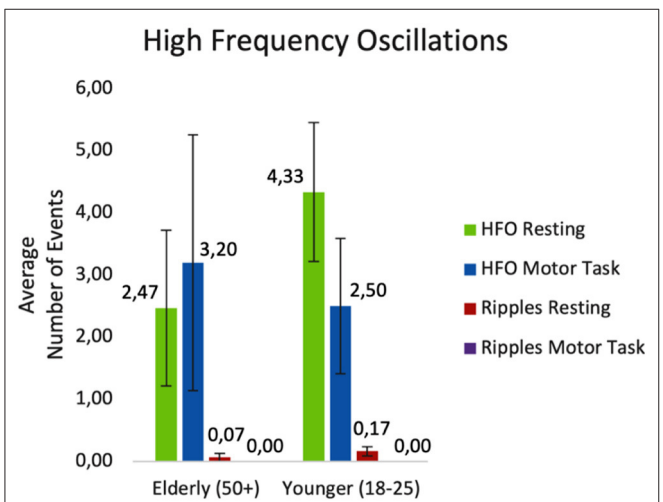

FIGURE 5 | Average number of events detected while resting and during the motor task. HFO, ripples and unclear HFOs combined; Ripples, HFOs at 80–250 Hz.

The average number of detected events during resting and during the motor task are displayed in **Figure 5**.

Statistical analysis was based on the mean HFO rate of patients. The results of the semi-parametric repeated measures ANOVA revealed no significant difference for the factor age ($F_{(1,25.53)} = 0.09$; $p = 0.767$). There was also no significant difference in HFO rate between conditions ($F_{(1,Inf)} = 0.93$; $p = 0.334$). Finally, the results revealed a significant interaction effect between the two groups during the tasks ($F_{(1,Inf)} = 5.09$; $p = 0.024$). During the motor tasks, more HFOs were identified in the elderly group, while the opposite was the case during resting, with more events detected in the younger group of patients.

Difference of HFO Rate; Epilepsy Patients vs. Control Group

Regarding the events detected while patients rested, a *Mann-Whitney* test indicated that there was no difference in the number of ripples between the group of patients with epilepsy vs. the group without epilepsy ($U = 23.0$, $p = 0.661$, $r = 0.16$).

Even after including unclear HFOs the analysis, the *Mann-Whitney* test indicated that there was no difference between the two groups ($U = 27.5$, $p = 1.00$, $r = 0.0$).

No ripples were identified during the learning trials of the fingertapping task. Regarding overall HFOs (*ripple* + *uHFO*), the *Mann-Whitney* test indicated that there was no difference between the two groups ($U = 26.0$, $p = 0.913$, $r = 0.04$).

HFO-Rate and Sleep

A Spearman's rank-order correlation was run to determine the relationship between the amount of sleep the patients had the night before the recording and the number of detected events, both while resting and during the motor task. There was no significant correlation between sleep and the number of detected

TABLE 4 | Diagnosed epileptic hemisphere and absolute HFO activity.

ID	Epileptic hemisphere*	HFOs – Total number	
		Left hemisphere	Right hemisphere
< 25 years			
004	Right	2	0
022	Right	4	7
023	Left	14	11
024	Right	5	5
028	Front	6	5
029	NFD	11	15
> 50 years			
002	Left	6	3
007	Left	0	0
037	NFD	1	2
039	NFD	0	1
042	NFD	13	8

** As diagnosed by the responsible physician; NFD, no final diagnosis.*

HFOs while patients rested ($r_s(9) = -0.002$, $p = 0.995$). The correlation between sleep and the number of detected HFOs during the motor task was also not significant ($r_s(9) = -0.300$, $p = 0.369$).

HFO-Rate and Age; Lateralization

Spearman's rho correlation coefficient was used to assess the relationship between the patients' age and the overall number of HFOs detected in the epileptic hemisphere, i.e. number of HFOs combined across the two conditions, motor task and rest. There was no significant correlation between the two ($r_s = -0.397$, $p = 0.436$). Furthermore, after correction for multiple comparisons there was also no significant correlation between the patients' age and the number of HFOs detected in the non-epileptic hemisphere ($r_s = -0.841$, $p = 0.036$).

To provide an overview about a possible hemispheric dominance of HFO activity, we created a table, displaying the epileptic hemisphere as diagnosed by the physician, as well as the total number of HFOs split up by their hemispheric occurrence. Events were merged across conditions of motor and rest, as there was also no significant main effect for condition. Furthermore, there was also no significant relation between the number of HFOs observed in the HD-EEG and the epileptic hemisphere diagnosed by the physician. However, when considering the data shown in **Table 4**, the relatively low numbers of HFOs must be taken into account.

HFO-Rate and Age; Motor Related HFOs vs. Resting HFOs

Spearman's rho correlation coefficient was used to assess the relationship between the patients' age and the number of HFOs detected during the motor task, and the number of HFOs detected during the resting phase, respectively. There was no significant correlation between the patients' age and the number of HFOs detected during performance of the motor task ($r_s = -0.358$, $p = 0.486$). There was also no significant correlation between the patients' age and the number of HFOs detected during the resting phase ($r_s = -0.522$, $p = 0.288$).

DISCUSSION

Because HFOs are intended to be used as a diagnostic marker for the identification of the seizure onset zone (SOZ) and ideally also for the epileptogenic zone in patients with epilepsy, we posed the question whether HFO occurrence on the scalp needs to take into account age as a confounding factor. We claim that examining the relationship between age and HFO rate might be crucial in developing an accurate diagnostic biomarker. Through the investigation of HFO rates in both elderly as well as young patients, we examined a possible change in HFO activity that comes with age. Therefore, we formulated the hypothesis that the HFO rate is increased in older populations and that it can be detected using scalp-EEG.

However, we were not able to replicate the general relation between HFO rate and age with the data collected in this study. HFO rate was not significantly elevated in an older population of patients across tasks. We may critically ask the question whether HFO occurrence is only elevated due to aging in prior research, or whether there are also factors of young age that contribute to HFO occurrence that were so far ignored. Our data showed an interaction of HFO rate by task, which we could interpret as a sign for age-related processing differences with respect to motor tasks. The difference between tasks in the older group is very small, while the younger group showed more HFOs during rest. Future studies with larger samples that are recruited with a broad distribution in age could shed more light on the age dependency in both the younger and older subgroup and assess these differences under different conditions and task requirements.

The sample is, however, still so small such that important moderators as the duration of epilepsy, medication, and localization of the seizure onset zone could not be considered in the analysis. Future studies with larger sample sizes, including also a critical number of healthy controls, are warranted in order to address this question.

Regarding patients' amount of sleep, we could not find a link between HFO event rate and the amount of sleep the night before the hospitalization. According to recent reports about influence of sleep on mean HFO rates, future studies need objective methods for the examination of sleep to rule out limitations like biases of self-disclosure. Additionally, increased pre-hospitalization nervosity that may influence sleep-pattern and therefore HFO activity should be considered.

Due to the small sample size and the heterogenous diagnoses it was not possible to perform an analysis by epilepsy type or epileptic focus. Nonetheless, we performed correlation between HFO rates found in the epileptic hemisphere as well as the non-epileptic hemisphere. This analysis revealed no lateralization effect for mean HFO rate, although a tendency for a significant correlation between age and HFO rate could be observed for

the non-epileptic hemisphere. However, this effect was not significant after correction for multiple comparisons and the sample for this sub-analysis is rather small ($n = 6$), as for the other patients there was no clear localization of the seizure onset zone available. The rather high correlation coefficients warrant replication of this analysis in a larger sample. Furthermore, as can be seen in **Supplementary Table 2**, there is no clear pattern related to the type of epilepsy in terms of HFO rate.

Our results might contradict previous findings, but limitations and challenges regarding scalp HFO detection need to be considered. In contrast to Nakano and colleagues (44), who found that in invasive EEG the latter part of single HFO is enhanced in older subjects, using scalp EEG we investigated the influence of age on the total number of events occurring when performing different tasks rather than the increase of isolated segments of single events. Thus, our study design differs with respect to both the recording method and the primary objective. Although we did not demonstrate an age effect, the study at hand provides relevant considerations of how age associated aspects as well as non-age associated factors may influence validity and therefore quality of studies in the field.

In the following we will discuss the special setting of HFO detection in the scalp EEG that might explain the non-replicability of a relation between HFO rate and age in our sample.

Signal to Noise Ratio

High- frequency somatosensory evoked potentials (HF-SEPs) have been the target of recent investigations examining focal epilepsy as a network disease rather than an isolated dysfunction of narrow cortical regions (55). It was found that these HF-SEP's had a significantly longer duration in the affected hemisphere compared to the unaffected hemisphere as well as compared to controls, therefore reflecting interictal functional impairments of the thalamo-cortical network. A study conducted by Nakano and Hashimoto (44) used invasive EEG to record somatosensory evoked potentials by median nerve stimulation. They found that the later part of HFO activity is increased in older patients. These invasive methods provide the advantage of a higher SNR, for example by minimizing artificial distortions due to cranial or facial muscle-movement. Additionally they show a higher sensitivity for small electrical signals arising from deep brain-regions. This explains the better identifiability of HFOs in the invasive EEG. In the scalp-EEG, a low background level of noise is indispensable for the identification of these correlates of small generators of neural signals (38). It is not always possible to keep the background noise level low, such that the identification of small events is less likely. In addition, artifacts may overlap with genuine HFOs. It has been claimed that the use of automatic detection would help to overcome this problem by valid identification of small events even in data with a higher background noise like it can be seen in scalp-EEG (56). Additionally, it has been assumed by previous studies that HFO-occurrence is in general rare in scalp EEG (38) which would be an alternative explanation of the lower HFO rates found in the actual study.

Epilepsy vs. No-Epilepsy

In the study at hand, we were unable to find significant differences in HFO rates between patients suffering from epilepsy and patients with no epilepsy diagnosis. It has been shown by previous studies that HFOs are positively correlated with epileptic spikes arising from structural brain changes like they are observed in patients suffering from epilepsy (28). The fact that invasive EEG is almost exclusively conducted in patients with severe epilepsy could partly explain why some studies with invasive recordings report a higher rate of HFOs compared to our study, which exclusively analyzed scalp-EEG. Notwithstanding that HFOs can also be recorded in healthy subjects, they are less prominent than pathological ones. This was shown by Kandel and Buzsáki (57) where stimulation of mesial brain areas like the thalamus induced neocortical ripples. Because the study at hand was conducted on patients admitted to the EMU for epilepsy clarification it is possible that we recorded data from patients with less severe forms of epilepsy resulting in lower rates of identified HFOs.

Furthermore, since the study procedure was conducted on the first day of hospitalization before drug tapering was initiated, an influence of medication on the occurrence of HFOs cannot be ruled out. Unless otherwise stated, all patients in the study responded to their medication and did not suffer any form of drug resistant epilepsy. Out of 11 patients 4 had no medication at all. For a detailed overview about patients' medication see **Supplementary Table 1**. Studies in intracranial EEG show that medication withdrawal increases the rate of HFOs (58). Another study found that HF-SEPs are modulated by antiepileptic drugs (59). This study found that antiepileptic drugs reduce the amplitude as well as the duration of HFOs of the affected hemisphere. The HFO suppressing effects reported by these studies might explain why we were not able to find a significant difference in HFO activity between groups. Also, controlling for medication and other medical conditions statistically was not possible due to the small sample size and must be considered a limitation of the study.

Sample Size & Power

The probability of being able to statistically detect a true effect depends on the statistical power of the study which in turn depends on the effect size and the sample size. Given the small average effect size reported in neuroscience in general and in EEG assessment in particular, the importance of an appropriate sample size cannot be stressed enough to increase reproducibility of studies (60).

Because relatively few studies investigated a relationship between HFOs and age in the scalp- EEG and the fact that almost no study offers information about the presumed effect size and how the sample size was calculated (61), there is nearly no data for orientation. To overcome the limitations of small and heterogenic samples multicentered studies are needed in the field of HFO research. This is very challenging due to missing standardized protocols for both, recording as well as for the analysis of data.

Limitations of Segment Duration Using Scalp-EEG

A limiting factor regarding segmental length chosen in the study at hand was the time expenditure related to the manual analysis of the HD-EEG data. Due to this as well as an a priori determined study-duration and the goal of screening as many different patients as possible, we decided to analyze shorter EEG-segments of different tasks. Striving for highest possible quality, the study at hand aimed to map a maximal representative section of the entire EEG of the respective patients. In order to do so care was taken to achieve a maximum of data quality as well as a minimum of artificial bias. To overcome these shortcomings, future studies using automatic detection are needed therefore enabling the analysis of longer EEG-segments with the same or even less time expenditure.

Pathological and Physiological HFOs

An important challenge of HFO research is the distinction of physiological and pathological HFOs. The idea of contrasting resting EEG with EEG during task performance in our study was set out to provoke physiological HFOs related to movement, and potentially distinguish them from pathological HFOs. However, it is still not possible to distinguish HFOs on a single-event basis and a very sophisticated design with provocative conditions might be needed to document increased HFO occurrence over regions that are functionally involved in execution of tasks and evoke physiological activity in the high frequency range.

Although we did not find significant differences in HFO activity between older and younger patients with respect to epileptic lateralization, it is noteworthy that the *p*-values in point 3.4 are rather small when comparing the non-epileptic hemispheres ($r_s = -0.841$, $p = 0.036$). There are 2 possible explanations for the actual *p*-values. On one hand, it could be that younger patients showed increased physiological HFO activity in the non-epileptic hemisphere compared to the older patients due to higher rate of neuronal activity. However, whether these HFOs were of pathological or physiological origin cannot be assessed in this study. On the other hand, it could be a purely coincidental finding due to the sample size. In favor of this theory would be the fact that, on close inspection of **Table 3**, there is no visible difference between the two hemispheres with respect to their HFO activity, which rather suggests against excessive physiological HFO activity. Although the Bonferroni correction provides some protection against false positives, it also poses the risk of creating false negatives. This should be taken into account when it comes to interpretation of the results.

The relevance of being able to distinguish between pathological and physiological HFOs also becomes apparent when it comes to the interpretation of the ANOVA. From a physiological point of view, an increased activity during motor tasks in older subjects could indicate an increased cognitive effort, whereas in younger subjects it could indicate a possibly higher consolidation activity in the resting phases associated with the maintenance of the numerical sequences of fingertapping. From a pathological point of view, higher HFO rates could be indicative of neurodegenerative processes in task associated areas in older subjects, with a decreased activity in younger patients.

However, the most likely explanation in this case is that it is a coincidental finding due to the rather small sample size.

Although this study included various conditions, it is not easily possible to distinguish physiological from pathological HFOs with this study design. Interestingly, especially HFOs recorded during sleep can be highly indicative in these respects, as recently shown (61). Specifically, HFOs recorded with invasive EEG during sleep were described to occur mainly in the hippocampus and occipital lobe, irrespective of the seizure onset zone or lesions related to epilepsy (62). We did not have HD-EEG recordings during sleep at our disposal. Future studies should investigate the possibility to record physiological high frequency activity during deep sleep. However, we investigated resting phases before and between our tasks regarding HFO activity. Although this is not a substitute for long-term recordings it still serves the purpose of capturing an interictal activity with increased signal-to-noise ratio.

CONCLUSIONS

In this study, we did not confirm a difference in HFO-rate between a young (<25 years) and older (>50 years) group of patients with epilepsy. Several reasons may account for the non-significant differences between age groups observed in the scalp-EEG and future studies should aim at a larger sample size, analysis of longer EEG segments, acquisition of sleep-EEG, testing under various conditions of rest and cognitive effort, and record under drug withdrawal. Because HFO detection in scalp EEG provides a cheap and non-invasive alternative to invasive EEG it is important that further studies investigate if an age dependent increase of HFO activity is associated with structural aging processes rather than being of epileptogenic nature. We deem it to be crucial to take into account biases such as age when introducing HFOs as a new biomarker in the diagnosis and management of epilepsy.

DATA AVAILABILITY STATEMENT

The datasets presented in this article are not readily available because of privacy issues. Requests to access the datasets should be directed to philipp.windhager@hotmail.com.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethik-Kommission für das Bundesland Salzburg: E/1755, 415-E/1806/4-2014, initial approval on 30/03/2014, latest amendment on 11/07/2016. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

YH: conceptualization, writing—review, editing, project administration, and funding acquisition. PW, AM, and YH:

methodology and writing—original draft preparation. PW and AM: validation, investigation, and visualization. AM: formal analysis and data curation. ET and AB: resources. YH, ET, and AB: supervision. All authors have read and agreed to the published version of the manuscript.

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SUPPLEMENTARY MATERIAL

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MINI REVIEW
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Remote Memory in Epilepsy: Assessment, Impairment, and Implications Regarding Hippocampal Function

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Studies of epilepsy patients provide insight into the neuroscience of human memory. Patients with remote memory deficits may learn new information but have difficulty recalling events from years past. The processes underlying remote memory impairment are unclear and likely result from the interaction of multiple factors, including hippocampal dysfunction. The hippocampus likely has a continued role in remote semantic and episodic memory storage over time, and patients with mesial temporal lobe epilepsy (TLE) are at particular risk for deficits. Studies have focused on lateralization of remote memory, often with greater impairment in left TLE, which may relate to verbal task demands. Remote memory testing is restricted by methodological limitations. As a result, deficits have been difficult to measure. This review of remote memory focuses on evidence for its underlying neurobiology, theoretical implications for hippocampal function, and methodological difficulties that complicate testing in epilepsy patients.

Keywords: remote memory, long-term memory, consolidation, autobiographical memory, episodic memory, epilepsy, seizures, hippocampus

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INTRODUCTION

Anterograde memory deficits reflect the inability to form new memories, while retrograde amnesia refers to the loss of prior learned information. These forms of memory dysfunction provide insight into the processes of memory formation, consolidation, and retrieval. Nearly 50% of people with epilepsy have anterograde and retrograde memory impairments (1) which are often multifactorial in etiology, due to underlying hippocampal pathology (2, 3), frequent seizures (4, 5), early age of onset (6, 7), and long epilepsy duration (8). In 1953, patient H.M. developed severe anterograde and moderate, temporally graded retrograde amnesia following bilateral mesial temporal lobe resection for refractory epilepsy (9–11). H.M. revolutionized our understanding of the hippocampus' role in memory formation and paved the way for studies of anterograde memory in epilepsy. Remote retrograde memory deficits in epilepsy patients, however, are not well-understood. Patients with remote memory deficits may learn new information but have difficulty recalling

events from long ago (12). The time point at which a memory is considered remote is ill-defined, but is typically ≥1 year (13). Patients with mesial temporal lobe epilepsy (TLE) are at particular risk for remote memory dysfunction (14).

Research in remote memory has focused on autobiographical and general semantic memory. Episodic autobiographical memory is the recollection of personal events (i.e., celebrating your 10th birthday), while semantic autobiographical memory refers to factual knowledge of your past (i.e., the school you attended). In contrast, general semantic memory is minimally influenced by personal experience. General semantic memory pertains to common knowledge of public figures and events, such as recognition of famous faces and awareness of news items.

ANATOMY OF REMOTE MEMORY

The hippocampus and medial prefrontal cortex are activated during functional magnetic resonance imaging (fMRI) studies of remote memory. The anterior hippocampus is engaged during autobiographical recall and event construction, while the posterior hippocampus is implicated in event elaboration and spatial memory (15, 16). The hippocampus is comprised of multiple subregions that support establishment of new memories ("encoding"). Initial input from the entorhinal cortex (EC) projects *via* the perforant pathway to the *Cornu Ammonis* (CA), specifically area CA3, where intrinsic recurrent connections form a memory trace (17). The projection from the EC to CA3 may occur directly, or indirectly *via* the dentate gyrus, which reduces interference between similar information (17). The EC and CA3 activate CA1, which outputs information back to the EC and subiculum (17). Finally, the EC and subiculum relay information to neocortical locations (17). There is conflicting evidence, however, regarding the duration of the hippocampus' role in memory retrieval, resulting in opposing memory consolidation theories.

Across species, impairment of recent memory with relative preservation of remote memory is evident in the setting of hippocampal damage, a pattern termed "temporally graded retrograde amnesia" (18, 19). In contrast, patients with damage to lateral and anterior temporal neocortex can be amnestic for remote events (20). Autobiographical memory, for example, is intact in patients with damage restricted to medial temporal structures but is impaired when the injury extends to neocortex (21). These findings suggest that the hippocampus becomes less important for declarative memory storage and retrieval over time, the basic premise of the "standard model of systems consolidation" (SMSC) (22). This model proposes that new memories are encoded in both cortical and hippocampal regions, but are stored in progressively strengthened cortico-cortical synapses, while the hippocampal trace fades with time. The hippocampus is no longer required for retrieval once neocortical storage is sufficiently established (23). The hippocampal-dependent stage lasts up to 1 week after encoding, while the new information is gradually integrated into pre-existing cortical networks (24). Recall further strengthens cortico-cortical connections, resulting in the full transfer of the memory from the hippocampus to the neocortex ("consolidation") (24). Retrieval of consolidated memories is independent of the hippocampus, predicting intact remote memory in patients with hippocampal lesions.

In contrast, the multiple trace theory (MTT) posits that spatial and context-specific details of an episodic memory are encoded in the hippocampus and remain stored there indefinitely, although some information is transferred to the neocortex (25). The entire hippocampal-neocortical pathway forms the consolidated memory trace. During each subsequent retrieval, the trace is reactivated, and a new, slightly different trace is established, so that over time, multiple related traces exist to facilitate retrieval. The MTT also emphasizes a distinction between episodic and semantic memory, in that both rely on the hippocampus for encoding, but general semantic memory eventually becomes independent of the hippocampus. While hippocampal traces contain contextual, spatial, and temporal details, cortical traces are semantic and context-free. Personally significant semantic memories, however, may have continued mesial temporal involvement.

The MTT predicts temporally graded retrograde amnesia for general semantic memory following hippocampal injury (26). Hippocampal damage would preferentially affect recent general semantic memories, as remote memories would be well-established in the neocortex. Yet, numerous rodent studies provide opposing evidence, with flat retrograde amnesia gradients for semantic memory after complete hippocampal damage (26–29). The MTT also posits that episodic memories become more strongly established in the hippocampus over time as the number of traces pertaining to that memory increases. Recent episodic memories, which have fewer memory traces, should be more disrupted by partial hippocampal lesions than remote memories (30). While the MTT places importance on the amount of damaged tissue, the specific structures affected may be the relevant factor. Several rodent studies, however, showed that remote memories were more vulnerable to partial hippocampal damage than recent memories, even when the precise location of hippocampal damage varied (27–29). Further, electrophysiologic unit recordings (31, 32) and calcium imaging (33, 34) in rodents showed a constant or decreasing number of place cells representing spatial memories, which contrasts the MTT's prediction that the number of activated cells increases when re-experiencing a specific context. Although much of the rodent literature supports the MTT, conflicting evidence surrounding the memory consolidation process remains.

Derived from the MTT, the trace transformation theory (TTT) (35) accounts for changes in memories over time, transforming from highly detailed to coarse representations. The TTT emphasizes the role of the posterior hippocampus and its connections to perceptual posterior neocortical regions in supporting finely detailed memories and the anterior hippocampus for remembering the overall "gist." The anterior hippocampus connects with anterior neocortical regions, notably the medial prefrontal cortex, where schemas represent common features across multiple events. These pathways are reactivated over the memory's lifetime, shifting to and from finely or coarsely detailed recollections. While detailed recollection will

always require hippocampal activation, schematic memory may be supported by the neocortex alone.

The MTT and TTT are supported by neurophysiological data from patients with medically refractory epilepsy. Intracranial EEG recordings demonstrated sharp wave ripples, oscillatory patterns supporting memory reactivation, in the hippocampi during recall of autobiographical memories (36). Ripple rate was higher during retrieval of remote memories compared to more recent events, supporting a continued role of the hippocampus. Ripples preferentially occurred in the anterior hippocampus, which the authors considered "compatible with [a] gist-like recollection strategy." With increasingly remote memories, ripple rate patterns associated with autobiographical events gradually became more similar to patterns of semantic retrieval, consistent with the theory that memory traces evolve over time to more general representations. Further, ripples correlated with cortical high frequency band activation, including posterior neocortex and medial prefrontal cortex, supporting the theory of recurrent hippocampal-neocortical interplay in these regions during autobiographical recall. Electrocorticographic data is consistent with functional MRI activation of hippocampal, medial frontal, and parietal regions during recall of remote autobiographical events (37).

REMOTE MEMORY DEFICITS IN EPILEPSY

Remote memory abilities in epilepsy patients could also help distinguish between these models. If the SMSC is true, remote memory should be relatively unaffected in patients with mesial TLE, although patients with neocortical epilepsy may be impaired. If the MTT/TTT is correct, patients with either mesial TLE or neocortical epilepsy may have impaired remote memory. The distinction may be blurred, however, in that focal epilepsy negatively impacts wide networks, such that mesial TLE may impair neocortical function. Further, the epileptogenic neocortical regions may lie outside of those supporting consolidation, such that remote memory could remain intact. Studies are limited by the inclusion of post-surgical epilepsy patients, in whom mesial and lateral structures are removed, and mixed TLE groups with either mesial or neocortical epileptogenic zones. Nevertheless, most studies demonstrated remote memory impairments in TLE (**Table 1**).

Autobiographical Memory in Epilepsy

Autobiographical memory was impaired in TLE patients compared to healthy controls, although it did not correlate with subjective memory complaints (55), which deserves further study. The Autobiographical Memory Interview (AMI) and Modified Crovitz Test were intact for personal semantics in pre-surgical TLE patients, the majority with mesial lesions, but memory for autobiographical episodes was impaired relative to controls (49). Similarly, subjects with mesial TLE, a subset post-surgical excision with lateral resections of unclear extent, had intact semantic autobiographical memory, but poorer episodic memory on the AMI than controls across all time periods (47). Pre-surgical TLE patients, the majority with hippocampal pathology, were impaired when generating friends' names (semantic autobiographical memory) on the Autobiographical Fluency Test, with the right TLE patients performing worse than controls when generating autobiographical events (46). Pre-surgical data largely support the MTT, in that hippocampal lesions were associated with impaired episodic remote memory. Data regarding semantic autobiographical memory, however, lend inconsistent support for the hypothesis that semantic memory is consolidated strictly in neocortex (46).

Variable autobiographical memory performance is evident post-anterior temporal lobectomy (ATL). Patients post-ATL had normal autobiographical event recall on the Autobiographical Fluency Test (43). In contrast, a small sample of post-left temporal lobectomy patients had impaired autobiographical memory for pre-surgical events (38). Pre- and post-surgical patients also had impaired memory for autobiographical events, in that memories contained impoverished perceptual detail (56). Reasons for variable results are unclear, but may relate to the laterality or extent of resection. One study included patients post-ATL, which removes neocortical and mesial structures, and patients post-selective amygdalohippocampectomy (SAH), which spares neocortex (56), but did not directly compare the two groups.

General Semantic Memory in Epilepsy

General semantic memory is consistently impaired in TLE, pre- and post-temporal resection. Like studies of autobiographical memory, data support the MTT/TTT, demonstrating a continual role for the hippocampus in retrieval of remote memory, but do not suggest that consolidated semantic memories are restricted to neocortex. Hippocampal ripples, for example, were of similar magnitude during general semantic and autobiographical episodic remote memory retrieval (36). Patients with TLE with either mesial or neocortical lesions had poorer recall and recognition for general semantic memory of public news items compared to healthy controls, extratemporal epilepsy patients, and primary generalized epilepsy patients (12). Pre-surgical left TLE patients, most with hippocampal pathology, had impaired recall and recognition of public knowledge, assessed by the Famous Faces, Famous Events, and Public Fluency Tests (46). Pre-surgical patients with TLE, most with mesial lesions, had poorer performance than healthy controls when naming famous faces and scenes, answering questions about news events, and indicating whether famous figures were dead or alive (49). Post-left temporal lobectomy, general semantic memory deficits were also evident when naming famous faces, providing knowledge about famous events and people, and recognizing names of short-lived television shows (38).

Lateralization of Remote Memory in Epilepsy

Evidence for lateralization of remote memory in epilepsy patients is mixed. Some data indicate no relationship between laterality of the seizure onset zone and memory for public news items (12) or autobiographical memory (47). Both left and right TLE groups were impaired, for example, when generating friends' names (46). Other data suggest lateralization of certain types of remote memory, with impaired autobiographical event

TABLE 1 | Remote memory deficits in epilepsy patients.

Study	Subjects	Type of remote memory tested	Retrograde memory tasks	Findings
Barr et al. (38)	Post-surgical: 6 LTL 6 RTL 6 Healthy controls	Autobiographical Public/semantic	Famous Faces Test (39, 40) Television Test (41) Goldberg-Barnett Remote Memory Battery (42)	LTL < RTL = HC
Bergin et al. (12)	Pre-surgical: 14 LTLE 19 RTLE 18 Left Ex-TLE 15 Right Ex-TLE 10 PGE 30 Healthy Controls	Public/semantic	Questionnaire assessing knowledge of public events	TLE < Ex-TLE = PGE = HC RTLE = LTLE R-ExTLE = L-ExTLE
Lah et al. (43)	Post-surgical: 15 LTL 15 RTL 15 Healthy Controls	Autobiographical Public/semantic	Australian Remote Memory Battery (ARMB)—Famous Faces and Famous Events (44) Public Fluency Test (PFT)—Famous Names and Famous Events (45) Autobiographical Fluency Test (AFT)—Names and Events (45)	Famous events: RTL < HC = LTL Famous names: LTL < RTL = HC AFT-Names: RTL < HC = LTL AFT-Events: LTL = RTL = HC
Lah et al. (46)	Pre-surgical: 15 LTLE 14 RTLE 15 Healthy controls	Autobiographical public/semantic	Australian Remote Memory Battery (ARMB)—Famous Faces and Famous Events (44) Public Fluency Test (PFT)—Famous Names and Famous Events (45) Autobiographical Fluency Test (AFT)—Names and Events (45)	Semantic Memory: LTLE < RTLE = HC Autobiographical: TLE < HC
Viskontas et al. (47)	Pre-surgical: 8 LTLE 5 RTLE Post-surgical: 6 LTL 6 RTL 22 Healthy Controls	Autobiographical	Autobiographical Memory Interview (48)	Personal episodic memory: TL < HC = TLE Personal semantic memory: TLE = TL = HC
Voltzenlogel et al. (49)	Pre-surgical: 19 LTLE 19 RTLE 35 Healthy controls	Autobiographical Public/semantic	Autobiographical Memory Interview (48) Modified Crovitz Test (50, 51) Public Events Test (52) Famous Scenes Test (53) Famous Faces Test (Denkova and Manning, unpublished) Dead/Alive Test (54)	LTLE < RTLE < HC

Ex-TLE, extra-temporal lobe epilepsy; HC, healthy controls; L, left; LTL, left temporal lobectomy; LTLE, left temporal lobe epilepsy; PGE, primary generalized epilepsy; R, right; RTL, right temporal lobectomy; RTLE, right temporal lobe epilepsy; TL, temporal lobectomy; TLE, temporal lobe epilepsy.

fluency in right TLE and deficits regarding public knowledge in left TLE (46). Reduced right hippocampal activation was evident in patients with right mesial TLE compared to controls during remote autobiographical event recall, not explained by hippocampal volume (37). In contrast, hippocampal ripples occurred preferentially in the left anterior hippocampus during autobiographical event recall, in patients with either left, right, or bilateral seizure onset zones (36). Overall, when an asymmetry was present, patients with left TLE were typically more impaired, as seen with tests of public knowledge (38, 46, 49) and autobiographical memory (38, 49). Whether these findings relate to the verbal nature of tasks is unknown. Object naming and semantic and phonological fluency correlated with general semantic and autobiographical memory, which could explain group differences (46), although not seen in all studies (12).

Contributing Factors to Remote Memory Deficits

Several studies examined the effects of seizure-related variables on remote memory. Epilepsy duration and history of generalized seizures did not impact memory for autobiographical or public facts and events (46). Nor was autobiographical memory associated with hippocampal sclerosis or temporal lobe resection (47). Further, a history of status epilepticus did not predict remote memory for public events (12). Data regarding the impact of antiseizure medications (ASMs), however, are conflicting. Post-temporal lobectomy patients taking ASMs had poorer autobiographical semantic fluency than patients not taking ASMs (43), and pre-surgical TLE patients taking ASM polytherapy had impaired autobiographical event fluency compared to patients taking monotherapy (46). The number of ASMs also negatively correlated with recognition of famous events (46). No correlation

TABLE 2 | Remote memory tests.

Test	Type of remote memory tested	Testing protocol	Time periods tested	Scoring
Autobiographical Memory Interview (AMI) (48)	Autobiographical	(1) *Autobiographical Incidents Schedule*: describe specific memories from past; three per time period (2) *Personal Semantic Memory Schedule:* knowledge of facts about past; three items per time period	Childhood Early adult life Recent events (past 5 years)	*Autobiographical Incidents:* 27 pts total (9 pts per time period) *Personal Semantics:* 63 pts total (21 pts per time period)
Galton-Crovitz Test (50, 51)	Autobiographical	Recall life events related to each of 20 high-frequency nouns (e.g., "holiday," "birthday") and estimate date of memory produced	N/A	0–3 scale for each memory produced
Autobiographical Fluency Task (45)	Autobiographical	Recall of personal events and names of acquaintances from each time period in 90 s	Preschool Primary school Secondary school Five years post-school Current	Number of items reported per time period in 90 s
Famous Faces tests (39, 40, 44)	Public/semantic	Identify faces of famous people in photographs from six decades, categorized by decade and difficulty. For each target picture, two non-famous faces are used as foils, matched to targets by age, sex, and time period	N/A	Number or percent of faces recognized
Famous News Events tests (44)	Public/semantic	Questions relating to prominent events categorized by decade	N/A	Number or percent of events recalled/recognized
Dead or Alive Test (54)	Public/semantic	Recognize faces of alive vs. dead famous individuals State year and cause (natural or unnatural) of death.	N/A	Number or percent correct
Transient News Events Test (60, 61)	Public/semantic	Recall and recognition questions related to transiently popular news events, categorized by hemi-decade	Events since subject was 10 years old, from 1961 to 2013 (updated version until 2016)	Number correct

NA, not applicable.

was seen, however, between the number of ASMs and recall of news events (12), although studies differed in testing procedures, types of epilepsy included, and degree of seizure control. Data regarding age of onset are also variable. Poorer memory for famous events correlated with earlier age of onset (46), but this was not seen in other studies (12, 47, 49). Effects of seizure frequency are mixed, as well. Patients with less frequent seizures had better memory for personal (5) and public events (5, 12) than patients with more frequent seizures. Seizure-free patients also had greater autobiographical semantic fluency (for the previous 5-year period) than patients with active epilepsy (43). Other data demonstrated no significant correlation between seizure frequency and memory for personal events, public events, or famous faces (49). Results may be limited, however, by difficulty in measuring seizure frequency. Future studies should consider the potential impact of interictal epileptiform discharges and psychiatric comorbidities.

While similar deficits in autobiographical memory were seen in subjects with or without surgical resection (47, 56), the impact of surgery has not been studied within individual subjects or in general remote episodic or semantic memory. Further, current studies may be limited by a lack of detail regarding the extent of neocortical resection (47). "Standard" ATLs are not standardized, in that variable amounts of neocortical and mesial tissue may be resected. The impacts of surgical approach and size of the resection are unknown. Comparison of performance post-ATL with more selective surgeries may help clarify the role of temporal neocortex and duration of hippocampal involvement in remote memory. SAH, however, can disrupt cortical and subcortical fibers in the approach to mesial structures. Depending upon the surgical approach, SAH could be expected to impair remote memory function in a similar manner as ATL (57, 58). Future studies may consider the effects of laser induced thermal therapy (LITT) on remote memory, as this technique can create more selective lesions within the mesial temporal lobe. Stereotactic laser amygdalohippocampectomy can preserve anterograde verbal memory, highlighting the importance of extra-hippocampal structures in memory performance (59). Postoperative studies may also be enhanced by including MRI quantification of the resected structures as a covariate.

METHODOLOGICAL ISSUES IN ASSESSING REMOTE MEMORY

Studies of temporal lobe damage provide conflicting support for the SMSC, MTT, and TTT. To understand variability across studies, we must consider the tasks used (**Table 2**) (39, 40, 44, 45, 48, 50, 51, 54, 60, 61). Several methodological factors limit remote memory testing, including a lack of control over the time and circumstances of encoding. Long-standing anterograde memory deficits may result in poor initial memory acquisition, which may falsely appear as remote, retrograde deficits. Further, the date of the event may not correspond to the time of acquisition, such

that the "age" of a memory may be uncertain. It is also difficult to control the frequency of memory rehearsal or re-exposure, which may allow easier recall or recognition. Tests of autobiographical fluency (45), for example, can include recurrent events.

Autobiographical memory is often complicated by emotional and contextual factors (62). Emotional events create vivid autobiographical episodic memories, often resulting in highly detailed "flashbulb" memories of the moment an event occurred. These emotionally-laden memories are more often rehearsed and more easily recalled, which could lead to an overestimation of remote memory abilities. Emotional memories also involve extratemporal regions, including frontal cortex, such that effects of TLE localization and lateralization are not straightforward. Third-party validation of self-reported memories may present challenges, as it may be difficult to find someone to corroborate the subject's responses, and the third party may not accurately remember events. It is difficult to establish response accuracy, and the detail required for a "correct" response is unclear.

Assessments of general semantic knowledge also pose several methodological issues. People who lived outside of the country in which the event occurred may not have been exposed to the material being tested. Exposure to news items may also vary based on interest (63). Further, questions regarding "recent" events will become outdated over time, requiring questionnaires to be updated frequently. Many tests assess material from the past several decades, of which younger subjects may be unaware. Related events or events of a similar nature may be easily confused. Lastly, some news items may be more prominent than others, appear in the news for greater lengths of time, or become popular again after several years, such that saliency may vary.

The Transient News Events Test (TNET) accounts for several of these methodological issues. It controls for saliency by using news items with a similar frequency of reporting, measured by the number of mentions in the New York Times Article Archive. Frequency of rehearsal is largely controlled by including events that had similar declines in the number of mentions over a 5-year period, allowing assessment of memories from restricted time periods. The variation in what can be considered a "correct" response is minimized, as the amount of detail required is clearly defined for each question. Finally, subjects are asked about events occurring when they were 10 years of age onwards, accounting for a possible lack of media exposure in early childhood.

Epilepsy poses additional challenges to remote memory testing. Early onset of epilepsy and resultant functional reorganization may have two consequences: anterograde and retrograde memory impairments may be difficult to differentiate, and different cognitive processes for encoding and retrieval may be used when compared to healthy subjects (64). Further, interrupted schooling may affect exposure to test items.

DISCUSSION

The literature lends insight into the brain regions involved in remote memory, though our current understanding of remote memory storage and retrieval remains limited. The exact role of the hippocampus is unclear, resulting in opposing theories of memory consolidation: the SMSC and MTT/TTT. The study of remote memory deficits in epilepsy can help to distinguish between these competing theories, as epilepsy may be associated with lesions of the underlying pathways. Data suggest a continued role for the hippocampus in remote general and autobiographical semantic and episodic memory and argue against the hypothesis that general semantic memory storage is restricted to neocortex. Studies are constrained, however, by methodological limitations of remote memory tests. We need improved, standardized testing for remote memory loss in epilepsy patients, and its inclusion in clinical neuropsychological batteries should be considered, as remote memory loss may be a source of disability not detected by current clinical testing. Further study of epilepsy-related factors and mechanisms of decline would help elucidate how seizures impair remote memory.

AUTHOR CONTRIBUTIONS

SR conducted the literature review and wrote the initial draft of the manuscript. KM contributed to the literature review and critical revision of the manuscript. WB and OD contributed to critical discussions regarding the topic. BL-M conceived of the manuscript and contributed to the literature review and drafts of the manuscript. All authors agree to be accountable for the content of the work.

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REVIEW
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Mechanisms for Cognitive Impairment in Epilepsy: Moving Beyond Seizures

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There has been a major emphasis on defining the role of seizures in the causation of cognitive impairments like memory deficits in epilepsy. Here we focus on an alternative hypothesis behind these deficits, emphasizing the mechanisms of information processing underlying healthy cognition characterized as rate, temporal and population coding. We discuss the role of the underlying etiology of epilepsy in altering neural networks thereby leading to both the propensity for seizures and the associated cognitive impairments. In addition, we address potential treatments that can recover the network function in the context of a diseased brain, thereby improving both seizure and cognitive outcomes simultaneously. This review shows the importance of moving beyond seizures and approaching the deficits from a system-level perspective with the guidance of network neuroscience.

Keywords: epilepsy, cognition, neural coding, information processing, place cells, population coding, phase precession

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INTRODUCTION

Epilepsy is a disorder of the brain characterized by an enduring predisposition to generate seizures and by the neurobiological, cognitive, psychological, and social consequences of this condition (1). Although seizures are an important part of the definition, the associated cognitive and behavioral impairments and learning and memory problems are also important determinants of quality of life (2, 3). The quality of life is highly affected in patients with epilepsy especially if they are in adolescence, affecting the patient's self-esteem and sense of coherence (4). Self-esteem is a main contributor to psychosocial wellbeing, personal reflection, and positive attitude (5), and coherence is the ability to recognize stressors as manageable and solvable (4, 6). In a 5-year follow up study, it was shown that sense of coherence decreased in adolescents with epilepsy. Adolescents who still had seizures showed a greater decrease compared to seizure-free teens, with no effect of seizure frequency on sense of coherence (4). Self-esteem was also decreased in adolescents with epilepsy, however, self-esteem was affected by the seizure frequency where higher seizure frequency was associated with lower self-esteem (4). This indicates that psychosocial wellbeing is affected in adolescents with epilepsy seen by the decrease of both self-esteem and sense of coherence.

Gauffin et al. (7) examined the experience of living with epilepsy and cognitive decline. Their study found out that cognitive decline is persistently present in adults with intractable epilepsy and living with epilepsy and cognitive deficits affected education, employment, self-esteem, social life, and future plans (7). The cognitive deficits are seen in both focal and generalized seizures. Focal seizures occur in a lateralized network in contrast to generalized seizures occurring in a widespread network encompassing both hemispheres (8), however similar cognitive impairments are seen in both epilepsies (9). Patients with focal epilepsy experience various cognitive impairments such as language abnormalities, executive dysfunction, attention deficit and long-term episodic and semantic memory deficits (9–11). Patients with generalized epilepsy experience the same cognitive impairments in addition to acquired knowledge deficits and long-term information processing and retrieval impairment (9, 12, 13).

A common pathophysiological argument for the mechanisms of the relationship between epilepsy and cognition is that the seizure and epileptiform discharges in the EEG directly injure neural networks that are the normal substrate for cognitive function. An alternative hypothesis is that the relationship is indirect; both the seizures and the additional morbidities arise from neural networks that have been disrupted by the etiology of the epilepsy e.g., single gene disorders, malformations of cortical development, or traumatic brain injury. Our starting position is that the action potential is the fundamental unit of information processing in the brain, and that sequences of action potential firing in neuronal populations over time are therefore considered to be mechanisms of cognition, as cognitive function is explicitly about information processing (14, 15). Our goal in the current article is to explore the latter hypothesis in detail, starting at the systems level physiology of brain function and relating this exploration to our understanding of epilepsy. We will describe these system level mechanisms in physiology (**Figure 1A**) and discuss how these mechanisms are changed or altered in brain diseases associated with epilepsy (**Figure 1B**), especially in the hippocampus and neocortex, which are important structures involved in memory.

RATE CODING

The firing rate of neurons in the brain is a mechanism of information transfer (15). For example, in motor neurons, the degree of muscle flexion depends on the number of action potentials per unit time (14). Tactile and texture perception in rodents is also, at least in part, a function of rate coding (16). In hippocampus and entorhinal cortex, rate coding is illustrated by place, time, and grid cells, respectively (17, 18).

Place cells are hippocampal pyramidal cells that fire when an animal visits a specific region of the environment: the cell's "place field" (19, 20). In any given environment, place fields cover the entire space to create a hippocampal cognitive map that is a representation of that space (19, 20). Repeated recordings of place cell populations have shown that the same cells are activated whenever the animal visits the same region in physical space, which suggests that the cells' representation is held in the network once the animal explores that specific field (21). This representation allows recollection of specific spaces and accurate navigation through the environment. This is further supported by the observation that lesioning the hippocampus results in loss of spatial memory (22). Similarly to place cells, time cells in the hippocampus fire at specific times in a task (e.g., the beginning, middle, or end) called time fields (23). These cells can be time locked to an external stimulus, like a tone, or intrinsically by a neural circuit or an oscillation (14, 15).

Grid cells are entorhinal cortical cells that provide activity-based maps of speed and direction in a certain environment (24). Grid cells fire in different locations in an environment forming a triangular grid. Recording different cells at the same location indicates that these cells have the same orientation to the environment (24, 25). It is important to note that the orientation of the grid relative to the environment is dependent on the hippocampal place cell map (20, 25, 26). Interestingly, the map formed by the grid cells is based on external cues, however the formed map persists even in the absence of these cues (24). Together with the place cell map, the grid cell map is believed to be part of the greater hippocampal cognitive map (20). Given what we know from work on place and grid cells, further experiments have shown that firing rate variations in CA3 place cells depended on signals from the lateral entorhinal cortex (24, 27). Lesioning the lateral entorhinal cortex impairs the hippocampal rate remapping upon changing the configuration of the environment (24, 27) suggesting that inputs from the entorhinal cortex are important for hippocampal rate coding in the formation of the spatial memory and cognitive map.

POPULATION CODING

Rate coding as measured in individual neurons is essential for information processing. However, neurons are functionally connected into a network and interactions between the neurons is also critically important (15, 28). This is known as population coding. Population coding increases robustness of network function and minimizes the effects of noise carried by an individual neuron, ensuring that the signal and processing times are not affected (15). For example, damage to one cell will not have a devastating effect on the information being carried since it is carried by many cells (28). Population coding is common in the nervous system and is illustrated in mammalian visual pathways (29), primary motor cortex activity in cats and monkeys (30), owl auditory cortex, cricket nervous system (31), and mice visual cortex (32–34). Neurons in the visual cortex in cats and monkeys and the auditory cortex in owls have shown the ability to synchronize their firing on a few milliseconds time scale through time cells (14, 35). Synchrony seen in the visual cortex of cats and monkeys takes place when the neurons are activated by one stimulus (35), and this synchrony is lost when the neurons are activated by two independent stimuli. Once two neurons synchronize to represent a certain stimulus, these two neurons always synchronize to represent the same stimulus and will desynchronize when representing two independent stimuli (14).

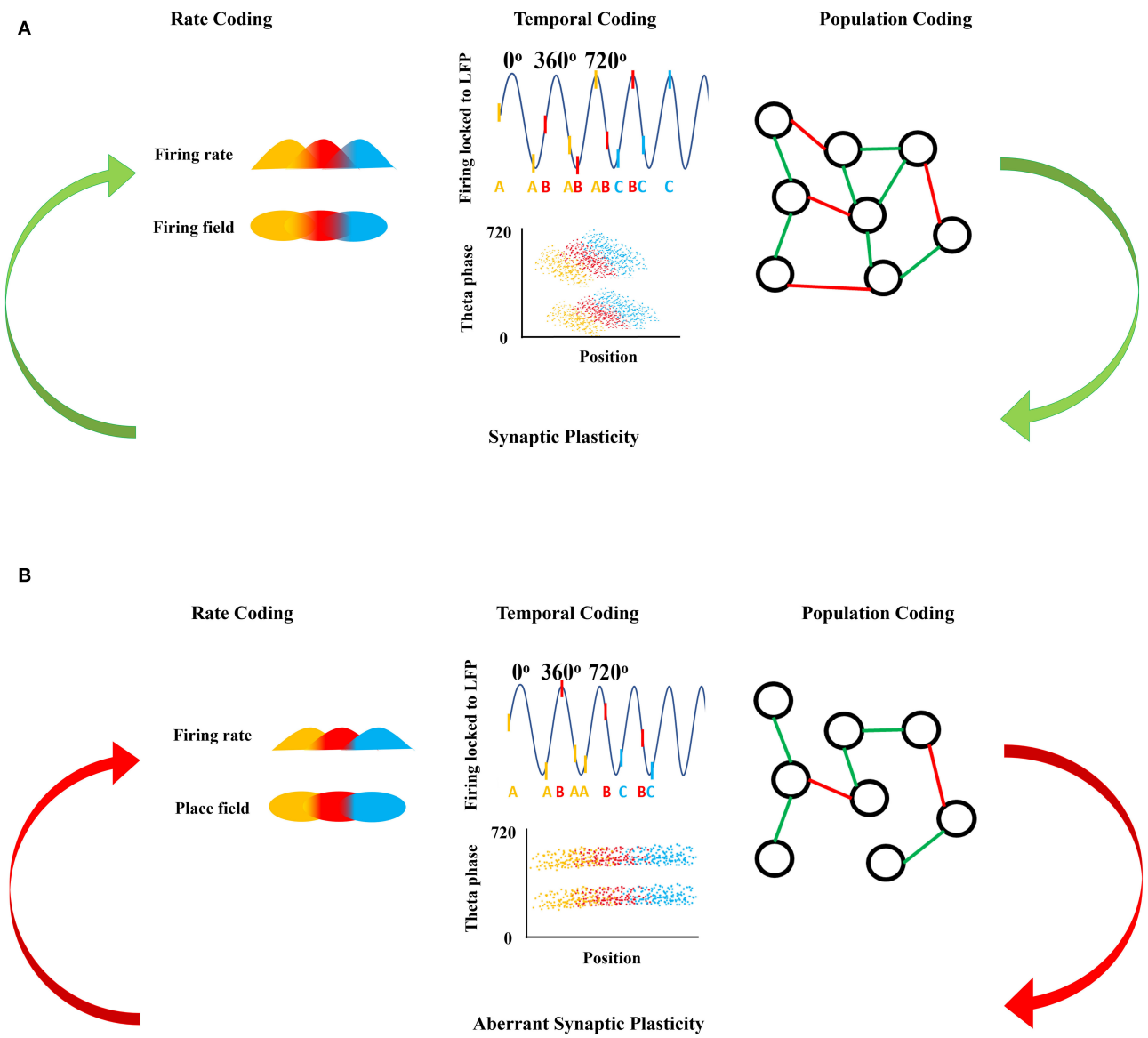

FIGURE 1 | Neuronal firing mechanisms and information processing. **(A)** Shows the normal information processing and synaptic plasticity feedback loop based on the neural coding mechanisms. Rate coding is shown as the firing rate in time (top) with respect to each firing field in space (bottom) coded by the same color. Temporal coding shows the action potentials as raster ticks riding on the LFP with respect to theta oscillation indicating theta phase precession with each successive peak of theta, with action potentials also represented as a letter at the bottom of the LFP representing firing from the same neuron over time (top). Over many LFP cycles, theta phase precession can be seen in the downward slope of the clouds of dots in the bottom panel, each representing an action potential from three representative neurons show in the top panel. Population coding shows the connections between neurons forming a population network, green representing an excitatory connection between two neurons and red representing an inhibitory connection between two neurons. Synaptic plasticity refines and is refined by these firing mechanisms. **(B)** Shows the disrupted information processing and synaptic plasticity feedback loop based on the neural coding mechanisms in epileptic brain. In epilepsy, rate coding is disrupted shown here as a decreased firing rate in time with respect to each firing field in space, with decreased overlap in the firing and place fields from each of the three color-coded neurons. Temporal coding is also altered; firing of the colored neurons with respect to theta oscillation is disorganized and there is an absence of theta precession as shown by a flat relationship between the clouds of action potentials from each of the three colored neurons. Population coding shows fewer connections between neurons forming a smaller population network, with potentially different proportions of positive (green) and negative (red) connections in the epileptic brain compared to controls in **(A)**. Aberrant synaptic plasticity occurs as a result of the aberrant firing dynamics.

Population coding influences information processing in mice visual systems. Whole cell recordings in layers 2/3 (L2/3) of awake mice have shown that the excitation/inhibition ratio changes based on the visual stimulus (33). Different studies revealed that patterns of excitation and inhibition are generated in response to various visual stimuli (36, 37). For example, as the stimulus contrast or size increases, the excitation/inhibition ratio decreases. This change in ratio is important for tuning and

sharpening of the information processing in the visual system (38) and is thought to be controlled by the somatostatin (SOM) neurons and parvalbumin (PV)-expressing cells. SOM neuron suppression was able to enhance the excitation and inhibition for size tuning, indicating that contrast and size in the visual system in mice depends on excitation/inhibition ratio and tuning of total synaptic input (33). PV cell manipulation was shown to modulate L2/3 pyramidal cells spikes in response to visual stimuli without affecting the cells tuning properties suggesting that PV cells create a connection between certain neuron types and specific computations during sensory processing (32, 39). Furthermore, the excitation/inhibition ratio for the same set of stimuli differs between anesthetized and awake mice and even differs between various behavioral states (33). This change in the ratio is influenced by the recruitment of SOM and vasoactive intestinal peptide-expressing (VIP) inhibitory cells in V1 in both wakefulness and alertness for instance (40–42). The Stabilized Supralinear Network (SSN) model proposes that cortical dynamics can change the excitation/inhibition ratio based on single neurons input/output supralinear relationships, strong recurrent excitation, and feedback inhibition (33, 43). This suggests that the dynamic change of excitation/inhibition ratio depends on the dynamic connections between different cortical regions and the feedback loops between the recruited regions. Further support to this suggestion was seen in experiments investigating the rhythmic activity across cortical layers. Population of excitatory neurons in the mouse's primary visual cortex expressed gamma band oscillation following layer-specific optogenetic stimulation (34). Importantly, rhythm-generating circuits in each layer were able to provide layer-specific excitation/inhibition balances hence influencing the information flow between cortical layers (34). These studies provide evidence that neuronal populations can be recruited through precise synchrony contributing to information processing at a system level.

Notably, place cells in the hippocampus are an excellent example of not only rate coding, but also population dynamics. We previously mentioned that the place cells fire whenever the animal is in a specific place field and these cells are distributed within the hippocampus in a way that covers the whole environment the animal occupies. This distribution within the hippocampus will cause a certain degree of overlap between the place fields, thus a population of cells will respond when the animal goes into the field rather than an individual cell since the cells in the hippocampus are receiving multiple sensory inputs to encode a multidimensional map (44).

Another interesting example of population coding is pattern separation. Pattern separation depends on the discrimination between two closely related places, episodes, or spatial configurations based on experience and can influence successful memory encoding. Pattern separation involves different brain regions where experiences are represented by neural populations. Notably, if the same population or neural pattern established during encoding is activated during retrieval, it can lead to a successful memory retrieval (45). For instance, both the posterior occipitotemporal cortex (OTC) and the hippocampus were recorded while participants performed item recognition tasks. Upon retrieval, both regions showed encoding-specific high frequency activity (HFA) where the strength of this activity was associated with enhanced retrieval, however the discrimination between similar items required a hippocampal activity (45) as the pattern separation mechanism is based on orthogonalizing similar input during encoding thus enabling the distinguishing between highly similar memories with minimal interference (46). Animal and human studies have shown that dentate gyrus (DG) and its projection to CA3 underlie the pattern separation process (47–52).

A final relevant example of population coding is working memory in the prefrontal cortex. Working memory is the temporary maintenance of information involving specialized components of cognition that allows retaining immediate past-experience, supporting new knowledge acquisition, solving problems, reasoning, and planning (53). Early models of working memory suggested that persistent firing activity of the neurons in the prefrontal cortex (PFC) throughout the delay phase of the working memory task was required to maintain information in working memory, however, due to the heterogeneity of neurons within the PFC, recent work has shown that the persistent activity of PFC can be weak or absent (54–56). This was seen during the delay phase of an image-sequence matching task in monkeys and humans. In this task, spiking activity in the PFC decreased during the delay phase of the task in monkeys, and BOLD signal on fMRI decreased during the delay phase in humans performing the task, thus challenging the persistent firing working memory model (57, 58). However, the spiking activity and the BOLD signal re-emerge during image presentation and testing period, indicating that, despite a decrease in firing rate throughout the task, the working memory information is maintained in the collective synaptic weights of populations of neurons in the PFC. The heterogeneity of the neurons allows different neurons to signal during different task events as well. For example, parvalbumin-positive neurons will respond to sensory cues and trial outcomes while somatostatin-positive neurons will respond to a motor action like licking (59). These neurons can fire together in working memory maintenance through population coding when a robust mnemonic stimulus is present (60) as seen in monkeys performing oculomotor delayed response and vibrotactile delayed discrimination, which are working memory tasks, while recording single neurons of lateral PFC (60).

TEMPORAL CODING AND OSCILLATORY FIRING

Oscillatory activity is divided into frequency bands as described in **Table 1**: infra-slow oscillations (0.5–1 Hz), delta (1.5–4 Hz), theta (4–8, 10 Hz), alpha (8, 10–12 Hz), beta (15–30 Hz), gamma (30–80 Hz), in addition to fast (80–200 Hz), and ultra-fast (200–600 Hz) ripples (61). Each band is thought to be related to specific aspects of cognition (**Table 1**). Both *in vivo* and *in vitro* experiments suggest that synaptic inhibition plays a role in generating neuronal oscillations through two different mechanisms, either through interneuron network activity or reciprocal excitatory-inhibitory loops (62). Theta, as previously

discussed, and gamma are two important readouts of the hippocampal function and function of connected regions.

Theta rhythm indicates a network that is actively involved in spatial navigation, working memory, and temporal coding (63, 64). It is important to note that theta oscillations and phase-locked neuron discharges with respect to theta oscillations are seen in theta non-generating regions like entorhinal cortex, perirhinal cortex, cingulate cortex, subicular complex, and amygdala (63, 65–67). Lesion or inactivation of the medial septum-diagonal band of Broca neurons recognized as the theta rhythm generators eliminates theta oscillation in all connected cortical regions (68) and leads to spatial and working memory deficits (69–71).

Gamma oscillations ride on top of theta in the hippocampus and dynamically couple hippocampal networks to specific behavioral demands (39, 72, 73). The gamma oscillation is thought to be a readout of information transfer from CA3 to CA1, where CA3 is influencing the activity of CA1 for hippocampal memory retrieval to underlie memory encoding, consolidation, and episodic memory retrieval. Movement sequences, memory encoding and formation, sensory processing and planned trajectories underlying spatial navigation are thought to be temporally organized through timing mechanisms seen through gamma oscillations (74, 75).

The rate of populations of neuronal firing is also modulated in time in respect to theta frequency. For example, in the hippocampus, temporal modulation is manifested as burst firing with bursts occurring at theta frequency (4–12 Hz). The fidelity of burst firing within theta, termed theta modulation, is important for phenomena such as phase precession, phase preference and hippocampal replay, which are believed to allow encoding of space with higher resolution than is possible in the absence of modulation. Phase preference refers to the phenomenon that neuronal firing in the hippocampus is often locked in time with respect to ongoing hippocampal inputs in the theta frequency of the local field potential (LFP). Specific cells fire preferentially at specific phases in the ongoing theta oscillation. For example, directly after the peak of the theta oscillation, PV+ basket cells in CA2/3 area fire at the same phase as pyramidal cells in CA3, but later than basket cells in CA1 (76). CA1 pyramidal cells preferentially fire at the trough of theta (77, 78). Phase precession describes the observation that place cells will discharge whenever the rodent is crossing a place field and this firing occurs at an earlier phase in theta with progressive theta cycle (79, 80). This phase precession is believed to be an important component of information processing. Theta-phase precession could be an indication of item-context associations through spike timing-dependent plasticity (80, 81) given that synaptic inputs need to be precisely synchronized within 5 ms so that EPSPs from different locations will be able to induce postsynaptic firing (15). Umbach et al. showed that neurons in the hippocampus and entorhinal cortex not only fire for space, but also for time. Interestingly, time cells also exhibited theta-phase precession during memory encoding, and the activity of these cells correlates with the use of temporal location during the retrieval phase of the task (82). Neuronal firing coordination with the LFP, like phase-locking and phase precession, offers a key glimpse at the relative timing of inputs in the LFP with outputs of the information processing as the neuronal firing, but neural oscillations are also readouts of synchronized behavior of the network and, as such, are on their own important mesoscale mechanisms of cognition, memory, and behavior.

Coordination between oscillations seen in the LFP or EEG can be an indicator of communication between different brain regions (83, 84). Coherence is a measure of this synchronization with values that range between 0 and 1. The higher the coherence, the more synchronized the regions are (84). The coherence value differs between different brain regions depending on the task performed, for example, theta coherence between hippocampus and striatum during periods of decision is high ($>$0.8) which indicate learning (84, 85). Not only theta coherence changes during a task, but also gamma as well. Attention tasks in monkeys have shown that gamma coherence increases between the parietal and prefrontal areas (86). Furthermore, CA1 can become coherent with the entorhinal cortex or CA3 through fast or slow gamma characteristics of the entorhinal cortex or CA3, respectively (87). Elevation of hippocampal-entorhinal cortex synchrony was shown to be important for declarative memory formation in epileptic patients performing a memorization task (88). This led to the hypothesis that synchronized brain activity in the gamma range might be an important indicator of controlled flow and routing of information (83, 89), because the rules guiding synaptic plasticity dictate that inputs will be most effective whenever they coincide with peaks of oscillatory network excitability (83). Finally, neural coherence alterations were associated with different disorders like schizophrenia, attention-deficit hyperactivity disorder (ADHD), Alzheimer's disease (AD), and temporal lobe epilepsy (TLE) (90–95) (**Table 2**).

FIRING DYNAMICS SUPPORT PLASTICITY

Temporal, population, and rate coding facilitate plasticity shaped through experiences that enable the brain to adapt to new information. These mechanisms underlie the careful coordination of information between synapses and neurons in the brain that is necessary to promote synaptic plasticity and ensure efficient flow of information between different brain regions required for cognition. In 1949, Donald Hebb postulated that synapse strength can change based on previous activity, which led to what we now know as long-term potentiation (LTP) and long-term depression (LTD), fundamental to network communication. LTP strengthens synaptic transmission through high frequency stimulation of synapses. The first stage of LTP depends on the NMDA and AMPA glutamate receptors (96, 97). The second and third stages of LTP depend on protein synthesis to maintain changes in synaptic strength (96, 97). Maintenance of LTP is essential for place cell stability (98–101). Even though neural plasticity is not a determinant of place cell spatial specificity, rats with neural plasticity deficits had unstable place fields upon revisiting the same environment (102, 103). This shows that neural plasticity is playing a role in

TABLE 1 | Frequency bands and cognitive processes.

Bands	Frequency (Hz)	Cognitive processes
Infra-slow	0.5–1	Show resting state networks (RSNs) in awake human subjects
Delta	1.5–4	Anticipation and predictive coding
Theta	4–8 (humans) 6–10 (rodents)	Spatial navigation, working memory, and temporal coding
Alpha	8, 10–12	Suppression and selection of attention
Beta	15–30	Involved in consciousness, logical/active thinking, focus, and stress
Gamma	30–80	Readout of information transfer from CA3 to CA1 for hippocampal memory retrieval. Show the temporal organization of movement sequences, memory encoding and formation, sensory processing and planned trajectories underlying spatial navigation.
Fast ripples	80–200	Show synchronous inhibitory postsynaptic potentials (IPSP) generated by interneuronal cell subpopulations
Ultra-fast ripples	200–600	Show synchronous inhibitory postsynaptic potentials (IPSP) generated by interneuronal cell subpopulations

TABLE 2 | Summary of cited clinical literature.

Study	Subjects	Experiment	Main findings
Spencer et al. (90)	Healthy and Schizophrenia Patients	Subjects responded whether an illusory square was present or absent in the trial along with EEG recording	• Abnormal phase-locking and abnormal phase coherence responses to the perception of an illusory visual stimuli in a Gestalt perception task that depends on neural synchrony • Abnormal neural circuit function may be an underlying cause of schizophrenia.
Herrmann and Demiralp (91)	Healthy and patients with ADHD, AD, epilepsy and schizophrenia	Gamma oscillations under various pathological conditions	• ADHD patients show an increase in gamma amplitudes • In Alzheimer's Disease (AD), there is a decrease in gamma response • In epileptic patients, there is an increase in gamma response which might be the readout of both cortical excitation and perceptual distortions • In schizophrenia patients, there is a decrease in gamma amplitude during negative symptoms, while there is an increase during positive symptoms such as hallucinations
Lega et al. (92)	Epilepsy patients	Recordings from hippocampal electrodes implanted in neurosurgical patients	• During successful episodic memory encoding there is an increase in the power of slow theta oscillations at 3 Hz • During successful memory encoding, there is a decrease in the fast theta hippocampal oscillation at 8 Hz
Barry and Clarke (93)	Children, adolescents, and adults with ADHD	Examine the resting-state EEG power and coherence, and event-related potentials (ERPs),	Different readouts that correlate with behavior and cognition: • Groups with high beta showed symptoms of increased delinquent behavior and reduced inattention, suicidal ideation, and physical problems. • Groups with elevated total power and theta and reduced alpha and beta showed fewer problems. • Groups with elevated slow wave activity and reduced alpha showed more impulsivity, inattention, and bad language. • Groups with reduced delta showed increased hyperactivity and ritualistic behaviors.
Wang et al. (95)	Healthy and AD patients	Recording resting eye-closed EEG signals followed by wavelet power spectrum and bicoherence of EEG analysis	• AD patients showed an increase in gamma and delta rhythms and a decrease in alpha power • The increase of the cross-frequency coupling strength between the beta/gamma and low-frequency bands in AD patients might be due to the disruption of GABAergic interneuron network showing an attenuated neuronal network

the organization of place cells and long-term maintenance of this representation.

The frequency of action potential timing matters; low frequency firing induces LTD, which decreases synaptic efficacy. LTD is also essential for memory formation as it counteracts the LTP to allow new memories to form. Recent evidence has shown that LTD may be involved in formation and maintenance of place fields (104), supporting previous experiments showing that a decrease in the expression of LTD impairs spatial memory retention and consolidation (105, 106). It is worth noting that plastic synapses can form positive feedback loops on the rate, temporal, and population coding mechanisms where this positive loop aids the mechanisms in refining and precisely timing the neuronal firing leading to a more efficient

information processing. Another form of plasticity known as short-term plasticity (STP) takes place on a millisecond to minutes timescale and depends on presynaptic calcium accumulation and vesicle depletion (107). This form of plasticity is thought to play a role in information transfer across synaptic connections, activity-dependent synaptic efficacy modulation, promoting synchronization and working memory (107–109). Careful coordination of the firing of populations of neurons in time supports appropriate short and long-term forms of plasticity that are critical for information processing, learning and memory.

EPILEPSY AND MEMORY

Cognitive impairments in people with epilepsy are extremely common and have a major negative influence on quality of life. Patients with focal epilepsy have shown to have a significant decrease in their quality of life compared to patients with generalized epilepsy and healthy controls, however, both focal and generalized epilepsy patients have a decreased self-esteem and increased anxiety compared to healthy controls (9). Identification of mechanisms of cognitive impairment is important as this will help to guide development of novel therapeutic strategies to improve outcomes. A major emphasis has been on studying the impact of seizures on cognition and exploring the epileptic encephalopathy hypothesis. Researchers have focused on the time of onset and frequency of seizures, however, there are few clear correlations between seizure characteristics and cognitive outcomes. Taken into consideration that cognitive functions are dependent on complex brain networks and both focal and generalized epilepsy groups share the same cognitive impairments (9), we can say that seizure location is less of a determinant of the cognitive impairments than an altered or dysfunctional network in the brain. This suggests that there must be other factors influencing cognitive outcomes in epilepsy. This assertion is supported by several lines of evidence. Studies assessing the effect of age of onset on cognitive impairment have shown that cognitive impairment already exists at pretreatment baseline in newly diagnosed children (3, 110–112). This led some researchers to question whether cognitive deficits could be present even before seizure onset (113) and this might suggest that the pre-existing impairments could be a result of the same dysregulation that underlies the seizures in the first place. Indeed, the need for special educational services prior to epilepsy onset is more common in children who were later diagnosed than those who weren't (114), suggesting that impairment in cognitive function may be present even before the first seizure. The best predictor of cognitive outcome up to 3 years after the diagnosis of epilepsy in infants is the initial cognitive profile, not any seizure or medication related factor. In adults, these impairments extend to deficits in visual motor tasks, mental flexibility, memory, reaction times, and attention (3, 115).

Others have studied the effects of the disorder duration on cognition, finding a negative correlation between the number of years of the disorder and brain volume (116, 117). This has been interpreted by some to mean that the length time since disease onset is related to the amount or significance of cognitive impairment. However, an alternate interpretation is that early onset of an epilepsy disorder is an indication of a more fundamentally dysfunctional network, leading to early development of recurrent seizures. Similarly, seizure frequency has been noted to be associated with a detrimental effect on cognition, with higher seizure frequency being correlated with lower performance on cognitive tasks and vice versa (9, 118). This was always interpreted to mean that seizures themselves were detrimental to cognition, rather than more frequent seizures being an indicator of a more abnormal neural network that underlies abnormal functioning during the interictal period manifesting as cognitive impairment. Hence, these impairments might not be due to age of onset or frequency of seizures, instead it could be attributed to the fact that children in this situation have a brain disease that presented earlier, and this difference in disease presentation may be an indicator of more severe network dysfunction, and more severe impairments. Further investigation of memory dysfunction in patients with epilepsy showed that people with epilepsy had significant deficits in both semantic and episodic autobiographical memory (9, 119, 120). This deficit was associated with young age at onset, more frequent seizures, and reduced working memory in early-onset epilepsy patients. In contrast, the same deficit was related to depression and lesion (3, 9, 120). The presence of both neurobiological and psychological factors suggests that information processing mechanisms might be altered (121).

Information processing through the mechanisms discussed in this review is shown to be altered in epilepsy and associated disorders. CA1 place cells are unstable in epileptic mice and undergo remapping a few weeks after pilocarpine-induced temporal lobe epilepsy (TLE). The number of place cells decreases, and the spatial tuning curve is less stable over time (122, 123). Prolonged recording over days from populations of neurons in CA1 and dentate gyrus has shown desynchronized interneuron firing between these two areas (124), which suggests that disruption of spatial coding is due to the loss of information processing control by interneurons. The desynchronized interneuron firing can affect the timing of the inputs being sent to the CA1. This was supported by the observation of theta rhythm temporal coordination loss in the dentate gyrus, where these neurons were firing at inconsistent phases of the CA1 theta rhythm (124). Spatial memory alteration was previously shown to be present even during the latent, seizure-free, period after either the pilocarpine-induced status epilepticus (SE) or early life seizures during the 1st weeks of life (39, 125, 126). These deficits were associated with a decrease in the power of theta oscillations (125). It is important to note that spontaneous seizures did not modify or affect any of the spatial deficits that were already present (125). Interestingly, Shuman et al. (124), found that place cell deterioration and place coding alteration occurred several weeks after pilocarpine induction, showing that the development of seizures is not solely responsible for place cell deterioration. Notably, place coding alteration, place cell deterioration, more dispersed place fields, and fewer place field responses were

also seen after silencing either CA3, entorhinal cortex or both (124, 127, 128).

In addition, we and others have shown dysregulated population coding in epilepsy models. *In-vivo* single-unit recording showed that CA1 pyramidal cells are functionally connected to other pyramidal cells and fire in a coordinated fashion during spatial memory tasks; this connectivity is altered in TLE where neuronal reactivation and synchrony predicts the behavioral outcome in a TLE model (129). Population coding functional connectivity is also crucial within the hippocampus and between the hippocampus and PFC to underlie spatial working memory (SWM) (130, 131). During a SWM task, the hippocampal-PFC network shows a distributed dynamic code, seen through temporally regulated firing within and between brain regions, which is needed to combine separate processes together to execute a SWM task (131). The coordinated firing of cells in time is important for several components like attention, decision making and long-term memory, which can predict task performance. The temporal modulation of populations of neurons predicted SWM accuracy in a delayed non-match-to-sample task in control rats and rats with a cortical malformation that, in humans, is an important etiology in epilepsy. Animals with cortical malformations showed deficits in hippocampal firing modulation in addition to decreased functional connectivity between neurons (131).

Furthermore, population coding and neural dynamics are important for pattern separation and this process has been shown to be altered in hippocampal injury and epilepsy. The pattern separation depends on a network spanning different brain regions other than hippocampus, like the dorsal medial prefrontal cortex (dmPFC), however the hippocampus and the parahippocampal cortex serve as a hub for this network (132), thus it is expected that a hippocampal injury will alter the network communication causing pattern separation deficits (47, 133, 134). TLE patients and amnestic mild cognitive impairment (aMCI) patients have pattern separation deficits, and this could be due to hippocampal dysfunction involving DG and CA3 (46, 135). Another reason could be due to the failure of separating similar information during encoding by the hippocampus, hence memories will not be accurately encoded or retrieved. Studies investigating aMCI and TLE patients have shown that aMCI patients have an excess activation of the DG/CA3 area in fMRI compared to control groups and this excess activation is correlated with poor performance on pattern separation tasks. The same poor performance was seen in TLE patients performing the Mnemonic Similarity Task (MST). TLE patients demonstrated poor pattern separation performance compared to controls, however, it is important to note seizure and hippocampal sclerosis did not affect the performance of patients in this task (46, 133). Following studies showing that TLE patients have spatial mnemonic discrimination impairment and that TLE mice have DG-dependent object location memory deficits (50, 136), Madar et al. (52) tested pattern separation in TLE patients and mice with TLE, and then used mouse brain slices to record the spiking patterns of single granule cells (GC) in the dentate gyrus. TLE patients performing object recognition-based MST had a significant deficit in identifying similar but not identical objects suggesting that TLE might be impairing the DG-dependent mnemonic discrimination. Similar deficits were seen in mice with TLE as the mice had a decrease in object-location mnemonic discrimination compared to control mice (52). Slice electrophysiology in the same mice utlized inputs mimicking the same recorded inputs during behavior, and indicated that the output spike-trains of GCs had a higher average correlation compared to input correlation, which signifies a deficit in pattern separation in mice with TLE. Different input ranges demonstrated decreased pattern separation and convergence in DG at multiple timescale levels (52). This shows the importance of population dynamics underlying spatial deficits and signals the importance of assessing functional connectivity.

Imaging and histological experiments showed that structural and functional connectivity were altered in TLE patients as well (3, 137). Histological changes have been observed in the amygdala, entorhinal and parahippocampal cortices in TLE patients (3, 138–142). MRI images investigating hippocampal sclerosis associated with TLE, found that in addition to hippocampus, atrophy is present in the adjacent mesiotemporal, temporopolar structures, and thalamus (3, 143–146), and this atrophy increases over time (147–149). Experiments investigating tissue microstructure and structural covariance indicate that structural connectivity was impacted in TLE. Diffusion tensor MRI showed a disorganization in fiber arrangement in temporolimbic and adjacent regions (3, 150–153). Structural covariance such as cortical thickness or gray matter volume was altered between the mesiotemporal and neocortical regions and within the corticocortical networks (154–156). Resting state functional connectivity revealed a deficit in network connectivity in TLE patients compared to healthy controls. TLE patients had a decrease in ipsilateral mesiotemporal networks connectivity and ipsilateral and contralateral hippocampi connectivity (3, 153, 157–159). This decrease in connectivity extends beyond the temporal lobes into the posterior cingulate, inferior parietal, and medial prefrontal cortices disrupting the default mode network (DMN) (160–164). These changes and deficits suggest that structural connectivity is impacted in TLE patients and that TLE is also associated with functional connectivity deficits and reorganization.

Early stage TLE patients experience functional connectivity deficits mainly in the ipsilateral hemisphere (162, 165) in addition to disturbed interhemispheric connections (3, 158, 166). However, in patients with generalized epilepsy, there is an increase in the interhemispheric connectivity in addition to reduced functional connectivity (167–171). fMRI studies investigating the network connections in epileptic brains showed an increase in functional connectivity within the temporal lobe, alongside a decrease between temporal and other regions. Also, there is a decrease in the connection probability between neighboring brain regions, known as the clustering coefficient, within the DMN (161, 172). This decrease in clustering coefficient as well as increased path length, i.e., distance between one node and another, was revealed to be associated with cognitive decline in patients with cryptogenic epilepsy and only seen in patients with cognitive decline (172–174). The decreased cluster coefficient within the DMN could underlie the language

impairment in patients with generalized epilepsy without focal brain damage. Gauffin et al. (175) conducted an experiment where patients with generalized epilepsy without focal damage performed a sentence-reading task while going through fMRI. Patients with generalized epilepsy took longer time to read both congruent (simple) and incongruent (complex) sentences compared to healthy controls with no reading time difference between congruent and incongruent sentences in the patients group which suggests that patients perceived both types as complex (175). BOLD fMRI indicated the activation of a left-lateralized frontotemporal network, anterior cingulate cortex and occipital cortex in both patients and controls upon reading both types of sentences (175). However, patients with generalized epilepsy had reduced DMN suppression compared to healthy controls (175). Further lack of suppression was seen in the left anterior temporal lobe and the posterior cingulate cortex, in addition to irregular activation of the right hippocampus proper and right parahippocampal gyrus (175). The reduced DMN activity suppression can be due to reduced functional segregation of the DMN in generalized epilepsy patients (170) where this can alter the balance between activated and deactivated neural networks hence disturbing the cognitive function (176, 177). Further evidence of network alteration in TLE patients was seen by Bernhardt et al. (178) upon analyzing hub nodes between controls and TLE patients. Hubs are also known as nodes that have multiple connections within a network with one central position and the connections formed by the hub nodes are essential for communication and network synchronization (179). Hub nodes in TLE patients were mainly located in the limbic and temporal association cortices instead of being evenly distributed between different lobes and this was thought to be due to connectivity disturbances between the temporolimbic and extratemporal neocortical structures (178) providing evidence that epileptic brains express decreased integration and enhanced segregation (172). It is also important to note that memory impairments are present in patients who don't show a lesion with MRI (180) which further supports the notion that cognitive impairments depend on the affected network rather than a structural lesion (9). These studies emphasize the necessity to move beyond the classical lesion model into a network approach which can provide several advantages by helping track or predict cognitive decline in epilepsy patients, improving diagnosis, and developing more accurate resection surgeries by targeting the areas where the hub nodes are mostly concentrated.

It is critical to note that experimental designs that induce an underlying disorder associated with epilepsy, but in which there are no overt seizures, and no other subclinical epileptiform activity was noted, show changes in information processing and behavioral deficits. Loss of function of sodium channels Nav1.1 associated with human epilepsy in CA1 can cause disruptions to place cells and spatial cognition without producing seizures (181). Nav1.1 knockdown in the medial septum causes alterations in temporal and rate coding in those neurons, and deficits in working memory that are correlated with the degree of LFP alteration in the hippocampus rather than seizure frequency (39, 182). Similar effects are seen in animals with a malformation of cortical development where no overt or subclinical seizures were noted. These animals have reduced fidelity of place cells, reduction in the magnitude of theta modulation, and disrupted population coding in addition to spatial and working memory deficits. The addition of induced seizures in this model did not make the behavioral deficits worse, indicating that the main contributor to the cognitive impairment was the underlying brain substrate and not seizures (183).

Notably, subclinical epileptiform activity or inter-ictal spikes (IIS) can disrupt cognitive function; however the number of spikes is not a reliable indicator of the associated cognitive impairment. Several studies have previously shown that patients with benign epilepsy with centro-temporal spikes experience IQ and school performance deficits (184, 185). These deficits were correlated with the frequency of IIS but not seizure frequency (184, 185). However, this may be related to timing of the IIS relative to ongoing cognitive processing, as the presence of the IIS may be an indicator that the brain is not in a state where it can be performing cognitive computations. Kleen et al. (186) investigated the effect of focal IIS on hippocampus in TLE. They showed that rats with unilateral intrahippocampal pilocarpine infusion developed hippocampal spikes that caused a response latency deficit in hippocampal-dependent operant behavior task, delayed-match-to-sample (186). However, the hippocampal spikes only altered the cognitive performance when they occur at the same time during memory retrieval; spikes occurring during memory encoding or maintenance did not affect the cognitive performance and overall IIS frequency during a trial was not predictive of accuracy during that trial (186). Similar results were seen in patients with refractory seizures performing Sternberg task, a delayed information task that depends on short-term memory processes, along with EEG recordings (187). Contralateral or bilateral to seizure focus hippocampal interictal epileptiform discharges (IED) during memory retrieval disrupted memory retrieval, and bilateral IED during memory maintenance was able to disrupt that process, however no effect was seen on memory encoding (187). These studies show that focal IIS and hippocampal IED are associated with disruptions in memory maintenance and retrieval only when they occur during the same time window as the memory processes. Taken together, this suggests that IIS/IED are indicators of disrupted network processing underlying cognition.

In addition to deficits in rate, temporal and population coding, plasticity deficits are also present in epilepsy, in accordance with the view that these neural coding mechanisms support plasticity. Kainic-acid induced status epilepticus (SE) model in rats shows a significant decrease in hippocampal LTP in addition to cell loss, and signs of hippocampal sclerosis (188). These rats also have deficits in the hippocampal-dependent novel object recognition spatial memory task that positively correlated with LTP magnitudes (188). These findings were also seen in the pilocarpine model where the mice showed a significant decrease in the hippocampal synaptopodin acting-binding protein in CA1 region which alters the ability of the neurons to express synaptic plasticity leading to a decrease in LTP induction in Schaffer collateral-CA1 synapses (189). STP and working memory are also altered in kainic acid-induced SE. Following kainic acid-induced SE, there was a decrease in STP, reduced LTP capacity, impaired

spatial learning, and increased inhibition in the dentate gyrus (190). STP was altered in a model with recurrent hyperexcitability leading to seizures during development (191), as well as a model with aberrant GABA signaling during development leading to frequent interictal discharges. Animals with frequent IDs in the developing PFC showed a decrease in attention, and sociability alongside these changes in STP (192). The growing evidence on neural networks and epilepsy shows that these disrupted neural networks are likely responsible for the cognitive impairments seen with the disease and that the underlying etiology is the cause of both the disease and coding impairments seen in epilepsy animals and patients as well. The corollary is that recovering neural networks toward normal has potential for recovering cognitive impairments (**Tables 3**, **4**).

THERAPEUTIC STRATEGIES

The main issue with finding the appropriate treatment is whether neural network function can be recovered even in the context of a diseased brain. Here we will discuss potential therapeutic approaches that might influence the neural network function.

Gene Therapy

Rett Syndrome (RTT) is a progressive neurodevelopmental disorder mainly affecting females in early childhood (196, 197). Development starts deteriorating at 6–18 months of age leading to neurological and neurobehavioral alterations and epilepsy (198). Loss of function mutations in the X-linked gene encoding the methyl-CpG-binding protein 2 (MeCP2) involved in transcriptional silencing and activation and RNA splicing modulation is thought to contribute to the pathophysiology of RTT (199, 200).

RTT is associated with significant behavioral abnormalities: motor discoordination and social interaction deficits as well as deficits in cognitive abilities like learning and memory (201–203). MeCP2 knockout mice show reduced neuronal activity in cortical and hippocampal areas (204) as well as deficits in LTP expression in the hippocampus (197). Epilepsy has been reported in 60–80% of RTT patients (205–207). Although children with RTT often have seizures, it is widely accepted that the main driver of the cognitive impairments is a function of the genetic cause. Deficits in LTP, reduction in neuronal activity and seizures indicate that behavioral and cognitive deficits extend to a network problem that involves several mechanisms underlying neuronal activity and plasticity.

Hippocampal place cells are impaired in RTT mice (203). Normally, place fields become refined as the animal-environment experience increases and are stabilized during memory consolidation in sleep. This process involves synchronous re-activation within high-frequency short time-scale windows, known as sharp-wave ripples (208), which is associated with synaptic plasticity transforming short-term memories into long-term ones (209). This process is disrupted in RTT mice as these mice show deficits in experience-dependent refinement of spatial information in addition to increased place cell baseline firing synchrony during sleep (203). Neural oscillations are also impaired in RTT. Organoids developed from stem cells of RTT patients, demonstrated individual neuron firing at a rapid and persistent rate, diminished or reduced gamma oscillation in addition to epileptiform-appearing spikes and high-frequency oscillations (210, 211). Rett mice show desynchronized and reduced theta oscillations during exploratory behavior (210, 212), underscoring impaired temporal coding underlying cognitive and behavioral deficits in RTT mice.

MeCP2 gene therapy has been shown to improve the survival and improve some behavioral deficits seen in RTT (196). Treated mice showed normalized gene expression in addition to better mobility and more exploratory behavior in the open field (213, 214), which could involve normalized place cell activity. This improvement was accompanied by a normalization of neuronal nuclear volume in MeCP2 transduced cells in the dentate gyrus (215). MeCP2 is a master transcriptional regulator of activity-dependent gene expression; recovering it may restore the brain's ability to respond plastically, thereby allowing the network to be in a state where it is ready to receive new information.

Rett syndrome is a very specific disorder whose pathophysiology seems to be directly related to MeCP2. Other causes of epilepsy are less straightforward and may require other gene therapy strategies. One such strategy is targeting the hyperexcitable granule cells in the dentate gyrus in TLE (216). Reducing granule cell hyperactivity *via* inhibitory chemogenetic receptors, DREADDs (CamKIIα-hM4Di), was able to normalize performance in the spatial object recognition task, reduce seizures and restore the dentate gyrus information coding process (216). Over-expression of the voltage-gated potassium channel Kv1.1 *via* lentiviral vector or AAV significantly reduced the seizure frequency in rats with focal neocortical epilepsy (FNE) or TLE, respectively (217). Evidence from behavioral and cognitive studies in epilepsy emphasize the need for a new gene therapy strategies. Cognitive and behavioral deficits vary among epilepsy patients, even patients with the same type of epilepsy as this could be due to different genomic factors (3, 218). Different genetic variants are associated with various comorbidities. For example, executive dysfunction was associated with catechol-O-methyltransferase (COMT), methylenetetrahydrofolate reductase (MTHFR) and BDNF in TLE and pediatric epilepsy (219, 220), memory impairment was associated with apolipoprotein E (APOE) and BDNF in TLE (221, 222), impaired working memory was associated with COMT and MTHFR in pediatric epilepsy (220), decreased information processing was associated with RE1- silencing transcription factor (REST) (219), and anxiety and depression were associated with BDNF and COMT (223). Further investigation of epigenomic, transcriptomic, and proteomic changes in epilepsy along with understanding the functional recovery mechanisms seen with gene therapy in RTT, TLE, and FNE at the level of rate, population and temporal coding will allow us to explore the possibility of treating diseased brains.

Environmental Enrichment

The efficacy of simple environmental enrichment (EE) strategies on improving cognition was first noted by Donald Hebb in 1947. He found that the rats he took home with him performed better on behavioral tasks than rats housed in the lab (224). This

TABLE 3 | Overview of cited preclinical research.

Study	Subjects	Experiment	Main findings
Austin et al., Oostrom et al., and Berg et al. (110, 111, 114)	Children with new-onset seizures	Behavior ratings, behavior questionnaires and school records	Cognitive impairment exists at pretreatment baseline, special educational assistance required for newly diagnosed children, cognitive impairment present before the first seizure
Brun et al. (127)	Rats	MEC lesion	Place coding alteration, place cell deterioration, dispersed place fields, and less place field responses
Schlesiger et al. (193)	Rats	MEC lesion	Loss of theta phase precession in CA1
Hales et al. (194)	Rats	Bilateral MEC lesions	Place field and phase precession deficits, impaired spatial precision and spatial stability
Hernan et al. (131)	Rats	Malformation of cortical development	Hippocampal-PFC network shows less temporal modulation and less connectivity, underlying deficits in SWM
Karnam et al. (126)	Rats	ELS	Reduction in coherence, information content, center firing rate, and field size of place cells, instability of place fields, and spatial learning impairment
Hernan et al. (191, 192)	Rats	ELS/ early life IID	Increased STP in the PFC, decreased attention
Lynch et al. (190)	Rats	Kainic acid-induced SE	Decreased STP, reduced LTP capacity, impaired spatial learning, and increased inhibition in the dentate gyrus
Suárez et al. (188)	Rats	Kainic acid-induced SE	Significant decrease in hippocampal LTP, cell loss, signs of hippocampal sclerosis, and spatial memory task deficits
Ewell et al. (123)	Rats	Kainic acid-induced SE	Decreased number of active place cells, decreased spatial tuning curve stability
Liu et al. (122)	Rats	Pilocarpine SE/TLE	Decreased number of active place cells, decreased spatial tuning curve stability
Chauviere et al. (125)	Rats	Pilocarpine SE/TLE	Spatial memory alteration took place during seizure-free period and decreased theta oscillations power
Tyler et al. (129)	Rats	Pilocarpine SE/TLE	CA1 hippocampal pyramidal cells functional connectivity, coordinated firing, neuronal reactivation and synchrony predicts the behavioral outcome
Lenz et al. (189)	Mice	Pilocarpine SE/TLE	Significant decrease in the hippocampal synaptopodin acting-binding protein in CA1 region, decreased LTP induction in Schaffer collateral-CA1 synapses
Shuman et al. (124)	Mice	Pilocarpine SE	Desynchronized interneuron firing between CA1 and dentate gyrus, theta rhythm temporal coordination loss in the dentate gyrus, place cell deterioration and place coding alteration
Clawson et al. (121)	Rats	Pilocarpine SE	Storage and exchange of information, theta and slow oscillations disruption
Lenck-Santini and Holmes (195)	Rats	Hippocampal sclerosis/TLE	Phase precession and temporal organization disruption

spurred Hebb's hypothesis that frequent pairing of neuronal firing leads to more efficient excitation in the future; that exposure to a more enriched environment during development critical periods might be influencing the behavior in adulthood. Hebb's observations were the first to connect environmental influences to plasticity. Today, EE paradigms involve exposure to different housing conditions that enable sensory, motor, and cognitive stimulation (225, 226).

EE has been studied in different neurological diseases like Parkinson's and Alzheimer's diseases. EE has been shown to slow cognitive decline in Alzheimer's disease (227). To investigate the effects of EE on epilepsy, it was shown that EE can reduce cognitive deficits, increase neural plasticity, improve motor coordination, and reduce the frequency of seizures (228). We will focus on the effects of EE on information processing mechanisms.

There is a strong link between EE, plasticity, and the mechanisms underlying plasticity. Housing young rats in an enriched environment for 30 days was shown to increase synaptophysin and post-synaptic density (PSD) in the cortex, hippocampus, thalamus, and hypothalamus (226). This suggests that the enriched environment was able to stimulate the formation of new functional synapses in these brain regions. Hippocampal gamma power increases during theta states in rats housed in enriched environments (229). This occurs alongside an increase in interhemispheric coherence of gamma oscillations after EE (229). Mice housed in EE conditions also had an increase in CA1 gamma oscillations (230).

EE also affects rate and population coding. Prolonged exposure to an enriched environment was able to increase the selectivity of CA1 place cells to a particular area in the arena in a way where fewer place cells are activated after brief exposure to a novel environment, along with an increase in global remapping efficiency and this was further supported by the increased expression of the activation protein Arc in CA1 and

TABLE 4 | Main takeaways for the pre-clinical sections and associated clinical relevance.

Coding	Physiology		Epilepsy	
	Preclinical	**Clinical**	**Preclinical**	**Clinical**
Rate	• Place cells fire when an animal visits a specific place field • Time cells fire at specific times in a task called time fields and they can be time locked to an external stimulus • Grid cells provide activity-based maps of speed and direction in a certain environment and fire in different locations in an environment • Place and grid cells map are part of the greater hippocampal cognitive map • Inputs from the entorhinal cortex are important for hippocampal rate coding in the formation of the spatial memory and cognitive map • Selective disruption of the theta rhythm power correlated with spatial component of the non-verbal correlates of episodic-like memory task	• Time cells fire at specific times in a task called time fields and they can be time locked to an external stimulus • Inputs from the entorhinal cortex are important for hippocampal rate coding in the formation of the spatial memory and cognitive map	• Place cell misfiring • Loss of accurate spatial navigation • Lesioning the hippocampus results in loss of spatial memory • Lesioning the lateral entorhinal cortex impairs the hippocampal rate remapping upon changing the configuration of the environment • Time and grid cells deficits	• Time cells firing deficits • Disruption of entorhinal cortex inputs • Spatial memory deficits
Temporal	• The rate of populations of neuronal firing is also modulated in time • Temporal modulation is manifested as burst firing with bursts occurring at theta frequency in the hippocampus • Theta modulation is important for phase precession, phase preference and hippocampal replay • Phase precession is important for information processing. • Theta-phase precession could be an indication of item-context associations • Selective disruption of theta coordination across CA1 and the DG correlated with temporal component of the non-verbal correlates of episodic-like memory task	• The rate of populations of neuronal firing is also modulated in time • Neurons in the hippocampus and entorhinal cortex fire for space and time • Time cells exhibited theta-phase precession during memory encoding • Time cells activity correlates with the use of temporal location during retrieval phase of free recall task	• Loss of phase precession • Temporal modulation deficits • Item-context association deficits	• Loss of time modulation of neuronal firings • Theta-phase precession deficits • Temporal location alteration in free recall task
Population	• Neurons are functionally connected into a network • Population coding increases robustness of network function • Place cell populations will respond when the animal goes into the field • Dentate gyrus (DG) and its projection to CA3 underlie the pattern separation process • Working memory in the prefrontal cortex depends on population coding	• Pattern separation involves posterior occipitotemporal cortex (OTC) and the hippocampus • Dentate gyrus (DG) and its projection to CA3 underlie the pattern separation process • Working memory in the prefrontal cortex depends on population coding • BOLD signal on fMRI decreases during the delay phase of image-sequence matching task in humans • BOLD signal re-emerge during the image presentation phase of image-sequence matching task • Working memory information is maintained in the collective synaptic weights of populations of neurons in the PFC.	• Loss of functional connections • Decreased robustness of network function • Loss of place cells firing accuracy • DG aberrant CA3 influences • Working memory deficits	• Early stage TLE patients experience functional connectivity deficits in the ipsilateral hemisphere and interhemispheric connections • Patients with generalized epilepsy have an increase in the interhemispheric connectivity but reduced functional connectivity • Decreased cluster coefficient within the DMN underlies the language impairment in patients with generalized epilepsy without focal brain damage • Reduced DMN activity suppression can alter the balance between activated and deactivated neural networks and disturb cognitive function • Hub nodes in TLE patients were mainly located in the limbic and temporal association cortices instead of being evenly distributed between different lobes • Memory impairments are present in patients who don't show a lesion with MRI

dentate gyrus (231). This might suggest that the exposure to an enriched environment might be changing how place cells process information by recruiting more or new populations of neurons leading to a more efficient population coding mechanism.

Due to the various effects of EE on these important mechanisms, interest has been growing in investigating the effects of EE on epilepsy. Exposing rats with absence epilepsy to an enriched environment resulted in fewer seizures in adulthood, reduced seizure frequency, and reduced anxiety levels in adulthood (232). The beneficial effects of EE were also seen in TLE rats in the lithium/pilocarpine model. EE was able to alleviate depression and hyperactivity in addition to restoring theta LFP power in the CA1 region (233). The positive effects of EE were further seen in rats with malformation of cortical development (MCD). Rats with MCD had a disruption in their fine spike timing and place-modulated rate coding in CA1 region, which was improved upon with EE exposure (234).

These studies show that EE have a positive impact on rate and population coding. This is important as these processes are disrupted in epilepsy where these are essential for information processing and plasticity. This opens the door for future investigations on how EE can possibly modulate the brain network in ways that make it less susceptible to insults and improves outcome in patients with epilepsy.

Brain Stimulation

Brain stimulation is another therapeutic option for improving cognitive deficits associated with a variety of neurological diseases. Brain stimulation can either activate or inhibit the brain activity in a specific region which gives the ability to modulate cognitive functions. Various types of brain stimulation exist, deep brain stimulation (DBS) is an invasive technique that involves direct implantation of electrodes in the brain while transcranial magnetic stimulation (TMS) is a non-invasive technique that uses magnetic fields applied to the head (235). Brain stimulation techniques have been mainly studied in Alzheimer's disease (AD) and Parkinson Disease (PD).

DBS was tested in AD for the first time in 1984, and while this study did not show any memory or cognitive improvements, it was able to partially stop the left frontal lobe deterioration (236). In 2010, DBS went into phase I trial to investigate its effect on AD patients, and it was shown that after DBS of the fornix/hypothalamus, the patients had improved memory, reduced cognitive decline, enhanced mental state and social performance in addition to increased hippocampal volume (237–239). Further experiments exploring DBS and AD took place after this trial, and the experiments showed the positive effects of DBS on stabilizing cognitive performance (240), influencing cognitive function and disease progression depending on the disease stage and brain region being stimulated (241). For example, nucleus basalis of Meynert (NBM) DBS had a positive effect on sensory gating of auditory information into memory (242). Repetitive TMS (rTMS) was also applied for AD patients. rTMS delivers trains of pulses at the same intensity over a period of time. It mainly uses high frequency ($\geq$5 Hz) for cortical excitability, low-frequency ($\leq$1 Hz) for cortical inhibition or theta-burst stimulation (TBS) (243). Several trials have shown that rTMS enhanced cognitive function in AD patients when applied to the bilateral dorsolateral prefrontal cortices (DLPFCs) (244–247). Animal studies also investigated the effect of DBS on AD. Acute fornix DBS was able to improve learning and long-term memory in the triple transgenic AD mouse (3 $\times$ Tg) model (248). Also, bilateral intermittent NBM DBS enhanced and maintained spatial memory tasks in AD rats (249). Similar results were seen with single rostral intralaminar thalamic (ILN) DBS, in addition to preservation of dendritic spine density in the mPFC and hippocampus and enhanced expression of PSD-95 (250).

In PD, bilateral subthalamic nucleus (STN) and internal globus pallidus (GPi) DBS was able to significantly reduce dyskinesia and improve motor symptoms with long-term benefit (251–253). Additional studies have shown overall improvement in quality of life and continued efficacy in patients that lasted more than 10 years (254, 255). Although most studies agree on the positive effects of DBS on motor function and quality of life, there is contradictory evidence on the positive effects of DBS on cognition and attention in PD patients. Some studies have found that PD patients continued to experience PD-associated declines in executive function, visuospatial reasoning and memory, and verbal memory after DBS (256–258). However, other studies have shown that DBS groups performed better than control groups in memory functions and visuospatial tasks (259, 260). The contradictory results seen with DBS on cognition in PD patients could be due to the stimulated brain regions and using on paradigm for all patients. STN and GPi are the most studied regions in PD due to their importance in dyskinesia and motor coordination, however these regions are not directly involved in memory *per se*.

DBS is used for epilepsy patients to control and manage refractory seizures; however, DBS may also be beneficial for the cognitive deficits seen in the patients. Ezzyat et al. developed a subject-based approach to investigate the effect of DBS on memory facilitation if performed in a timely manner. Taking into account the disrupted memory network in epilepsy patients, interfering at the right time can reverse the dysfunctional activity of memory encoding. The team was able to differentiate low from high encoding states which indicate neural activity and either stimulating a single medial temporal lobe (MTL) structure like hippocampus or structure involved in memory encoding like prefrontal cortex in the learning session (261). Studies stimulating a single MTL region had contradictory conclusions, indicating both memory facilitation (262, 263) and memory disruption (264, 265). Interestingly, the stimulation was able to increase the encoding-state and memory recall when performed during low-encoding states (261) and this suggests that the accurate stimulation of a single MTL structure or a region involved in memory encoding can reverse the deficits if done at a specific time of memory process. Pilocarpine rats showed a decrease in hippocampal theta power and percentage of time oscillating in theta (266), however, continuous stimulation through Barnes maze task or pre-task stimulation of the medial septum at 7.7 Hz was able to prevent theta oscillations reductions, improve spatial navigation and search strategy during the task. This cognitive improvement was accompanied by significant increase in seizure threshold in these rats. This shows that

theta stimulation of the septum has potential to rescue cognitive impairments and increase seizure threshold, further supporting a mechanistic link upstream of both of these symptoms of epilepsy (266, 267). The same stimulation paradigm was used with rats after a traumatic brain injury (TBI) and it was shown that these rats had improved spatial learning and object exploration in addition to increased hippocampal theta oscillations (268). Taken together, these data show that neuronal stimulation approaches may be effective in restoring normal network function and improving cognition broadly.

Interneuron Implantation

Interneuron implantation is another possible treatment that can potentially recover the network function given the importance of interneurons in balancing the inhibition-excitation, controlling gamma and theta oscillations, and sharp wave ripples in the hippocampus. Interneuron precursor implantation into the prefrontal cortex of Pten mutant mice, an autism mouse model, was able to reverse the social behavior deficits seen in these mice; however, the implantation did not normalize baseline and social interaction-evoked EEG signals, but did modify inhibitory signaling in the PFC, underscoring a complex relationship between etiology and circuit restoration underlying behavioral improvement in disease (269). Interneuron implantation has been shown to be beneficial in epilepsy as well. Implantation in TLE, absence epilepsy, and generalized epilepsy models in rodents was able to increase seizure threshold, reduce seizure frequency and duration, reduce network excitability, and improve behavioral deficits (270–273). Implanting interneurons derived from human induced pluripotent stem cell (hiPSC) into the hippocampus of TLE rat model was able to reduce spontaneous seizures frequency after status epilepticus (274–276) which shows translational significance from rodents to humans. In addition to reducing seizures frequency, there was a decrease in the aberrant mossy fiber sprouting, and improved cognition and mood. The implanted rats showed an improvement in hippocampal dependent tasks like object recognition and improvement in pattern separation and novel object recognition (276), which suggests that the implantation might be recovering the communication between different regions or reactivating the DG/CA3 connections required for pattern separation. Integration of interneurons into the CA3 network may be how the new interneurons are affecting the network. region of the hippocampus of epileptic mice was able to improve the working memory in Y-maze test and spatial memory in water maze, however both tasks depend on the PFC (270, 274, 277). This raises the question of how locally implanted interneurons can enhance tasks that are dependent on different brain regions as well. Given the crucial role for interneurons in the timing of the action potential firing, these local connections are likely refining the signal from hippocampus to the PFC. Interestingly, MGE implantation was able to increase memory precision in mice with traumatic brain injury (TBI) as well. Implanted mice performed better in object location task and contextual fear memory where both tasks depend on hippocampus and hippocampal interneurons, respectively (278). Based on these data, GABAergic interneurons transplants may be a promising therapeutic approach for different diseases, however, further investigations are needed to determine the right time and location of implantation for the different investigated diseases.

CONCLUSION

In this review, we addressed the role of neuronal dynamics in supporting proper cognition, learning and memory, and discussed how these dynamics are altered in epilepsy. The data suggest that cognitive impairments seen in patients with epilepsy and preclinical models of epilepsy are likely due to plasticity changes, alterations to neuronal coding regimes, desynchronization, and functional connectivity disruptions from the effect of underlying etiology, rather than seizures themselves. Although we accept that the seizures could also have some negative impact on network behaviors, we strongly argue that the seizure effect is very small when compared to the etiology effect. We therefore suggest that these deficits should be approached from a systems neuroscience perspective, while being informed by mechanisms needed for normal cognitive function and development in a dynamic experience-dependent and plastic network. Importantly, this calls us to move beyond seizures into network science that is guiding possible treatments and defining new pathophysiology. This might help advance the epilepsy research forward and open the door potentially to answer unsolved questions in the field.

AUTHOR CONTRIBUTIONS

MK, AH, and RS: draft manuscript writing and editing. All authors reviewed the final draft manuscript. All authors contributed to the article and approved the submitted version.

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Emotional Word Processing in Patients With Juvenile Myoclonic Epilepsy

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Objective: According to Panksepp's hierarchical emotion model, emotion processing relies on three functionally and neuroanatomically distinct levels. These levels comprise subcortical networks (primary level), the limbic system (secondary level), and the neocortex (tertiary level) and are suggested to serve differential emotional processing. We aimed to validate and extend previous evidence of discrete and dimensional emotion processing in patient with juvenile myoclonic epilepsy (JME).

Methods: We recorded brain activity of patients with JME and healthy controls in response to lexical decisions to words reflecting the discrete emotion fear and the affective dimension negativity previously suggested to rely on different brain regions and to reflect different levels of processing. In all study participants, we tested verbal cognitive functions, as well as the relationship of psychiatric conditions, seizure types and duration of epilepsy and emotional word processing.

Results: In support of the hierarchical emotion model, we found an interaction of discrete emotion and affective dimensional processing in the right amygdala likely to reflect secondary level processing. Brain activity related to affective dimensional processing was found in the right inferior frontal gyrus and is suggested to reflect tertiary level processing. Psychiatric conditions, type of seizure nor mono- vs. polytherapy and duration of epilepsy within patients did not have any effect on the processing of emotional words. In addition, no differences in brain activity or response times between patients and controls were observed, despite neuropsychological testing revealed slightly decreased verbal intelligence, verbal fluency and reading speed in patients with JME.

Significance: These results were interpreted to be in line with the hierarchical emotion model and to highlight the amygdala's role in processing biologically relevant stimuli, as well as to suggest a semantic foundation of affective dimensional processing in prefrontal cortex. A lack of differences in brain activity of patients with JME and healthy controls in response to the emotional content of words could point to unaffected implicit emotion processing in patients with JME.

Keywords: discrete emotion, dimensional emotion, neuropsychology, juvenile myoclonic epilepsy (JME), implicit emotion processing

INTRODUCTION

A multitude of neuroimaging and electrophysiological evidence has been collected in recent years on emotional processing in the human brain (1). However, it is still not entirely clear which brain regions reflect emotional processing and what is the time course of such processing (2). Two main views provide evidence on emotional processing in humans. Discrete emotion theories (3–5) suggest that a limited number of discrete emotions (e.g., anger, fear, sadness) are evolutionary ingrained (3) and culturally universal (6). In contrast, dimensional emotion theories (7, 8) propose that a limited number of affective dimensions (e.g., valence and arousal) constitute the basis of emotional processing (9). Initially seen as opposing views, recent evidence is in support of the idea that discrete emotions and affective dimensions could both reflect emotional processing in humans (10, 11). A framework which is in support of this way of processing is provided by the hierarchical emotion model of Panksepp (5, 12, 13). According to this model, discrete emotions and affective dimensions are not seen as opposing views, but rather as describing different processes operating on different, neuroanatomically distinguishable levels (14). At a primary process level, discrete emotions are thought to originate in subcortical circuits, such as the periaqueductal gray (PAG). At a secondary process level, primary process level emotions are transformed into conditioned responses by classical and instrumental conditioning, which is served by limbic structures including the amygdala (15, 16). At the tertiary process level, neocortical structures such as the prefrontal cortex are thought to interact with the lower process levels by cognitive processes and to be shaped by socio-cultural demands clustering primary emotion information further into constellations of positive and negative affect (17, 18). Reading single words with emotional content has been reported to activate brain areas such as the hippocampus, parahippocampal gyrus, amygdala, anterior and posterior cingulate cortex, and orbitofrontal cortex (19–23). Briesemeister et al. (10, 11) reported electrophysiological and neuroimaging evidence for the processing of discrete emotion words reflecting high and low happiness but also for the processing of words reflecting the affective dimension of positivity in line with Panksepps hierarchical emotion model. The electrophysiological results indicated sequential processing of emotion information, with the discrete emotion happiness, affecting the early visual N1 component, and the affective dimension positivity, reflected in an N400-like component and the late positive complex. (10). Neuroimaging revealed limbic activity in the right amygdala for words reflecting the discrete emotion happiness, and activity in the prefrontal cortex such as the left and right inferior frontal gyri and left medial frontal gyrus for the affective dimension of positivity. These and other results strongly suggest that emotion processing relies on extended networks and might be altered if structures like the limbic system or the prefrontal cortex are impaired. This could be observed in certain neurological conditions, such as epilepsy in which patients have impaired emotion recognition (24–30). Juvenile myoclonic epilepsy (JME) comprises 5–10% of all epilepsies (31) and is one of the most common age-related idiopathic generalized epilepsies with a high reported genetic pre-disposition (32). JME-onset peaks between 14 and 16 years, usually presents massive myoclonic, generalized tonic-clonic and absence seizures (33–36). Executive functions are reported to be impaired in JME, comprising mental flexibility, inhibition of automated reactions, abstraction and categorization, planning and verbal fluency (37–40). Behavioral problems in patients with JME, such as poor social adjustment and impulsive personality traits, resembling patients with frontal lobe damage, are often observed (41, 42). The prevalence of psychiatric disorders of patients with JME varies between 35 and 49% and studies demonstrated increased mood, anxiety, and cluster B personality disorders (41, 43). Neuroimaging revealed subtle structural and functional alterations in thalamus and frontal cortex associated with cognitive, behavioral and emotional disturbances (44–47). Furthermore, recent studies reported morphological and functional alteration involving thalamo-cortical circuits, cerebellum, bilateral hippocampi, and cingulate, insular and occipital cortices (48–50). Thus, the clinical picture of patients with JME shows cognitive as well as emotional impairments.

In this prospective fMRI study on patients with JME and healthy controls we aimed to extend and to validate the previous reported dissociation of brain regions involved in discrete and dimensional emotion processing (11), by presenting words reflecting the negative emotion fear and the affective dimension of negativity. In line with the results of Briesemeister et al. (47) we hypothesized that the processing of words with high fear values differs from those with low fear values in the right amygdala and that the inferior frontal cortex shows increasing activity with increasing negativity of words. Furthermore, we expected general faster response times for words with high fear values compared to words with low fear values. In addition, we expected patients with JME and healthy controls to differ in the processing of words reflecting the affective dimension of negativity and that this differential processing is revealed by

differences in brain activity in the inferior frontal cortex, since neuropsychological and neuroimaging evidence showed altered executive functioning in patients with JME. Concerning the neuropsychological testing, we expected patients with JME to show deficits in several verbal and cognitive measures related to executive functions and to be at higher risk of experiencing psychiatric disorders.

MATERIALS AND METHODS

Participants

A total of 47 patients with JME were recruited in the Epilepsy Outpatient Clinic, Clinical Department of Neurology, Paracelsus Medical University, Salzburg, and were compared to 62 gender-, education- and age-matched healthy controls. Inclusion criteria for patients comprised the diagnosis of JME based on criteria of the International League against Epilepsy (51), above 14 years of age with willingness to participate in the project as well as informed consent obtained from patients or parents. Exclusion criteria for patients comprised the occurrence of any epileptic seizure <72 h prior to fMRI, neurological illnesses other than JME, structural lesions (if known from previous preliminary examinations), incompatibility with MRI investigation (e.g., metal implants, claustrophobia), pregnancy, and acute intake of Benzodiazepines. Exclusion criteria for healthy controls comprised individuals <18 years of age, previously known psychiatric and neurological illnesses or known structural lesions of the brain, incompatibility with MRI investigation, and pregnancy. Mean onset of epilepsy was at 14.23 years of age, mean epilepsy duration was 12.85 years. Three patients (6.2%) did not receive any antiseizure medication, 28 patients (58.3%) received monotherapy and 17 patients (35.4%) polytherapy. For a detailed sample description see **Table 2**. All participants gave written informed consent in accordance with the guidelines set by the local Ethics Committee.

Methods

Procedure

Psychiatric and neuropsychological assessments and functional magnet-resonance-imaging (fMRI) were carried out after all participants gave written informed consent. The test battery comprised a structured clinical interview on psychiatric and personality disorders, verbal intelligence, verbal fluency, verbal memory and reading speed. As the entire battery took about 2 h to complete, participants who were not able to complete it at once and were allowed to take part in two sessions. Additionally, participants completed structural and functional MRI. fMRI lasted about 1 h. fMRI was performed either before or after neuropsychological evaluation, or if participants wished to do so, on one of the following days. Healthy controls were compensated €100 for their participation, patients with JME did not receive financial compensation.

Neuropsychological and Clinical Tests

SCID I + II

The Structured Clinical Interview for Axis I disorders (SCID I) (52) and for Axis II disorders (SCID II) (53), based on the Diagnostic and Statistical Manual of Mental Disorders-IV, are considered to be the gold standard for a semi-structured assessment of clinical disorders and personality disorders, respectively. German versions (54) were used to assess DSM-IV Axis I and Axis II pathology.

Multiple-Choice Vocabulary Test

The Mehrfach-Wortschatz-Intelligenzttest (MWT-B) is a multiple-choice vocabulary test which serves as an estimate of crystalline verbal intelligence (55, 56). In this test the participants are asked to identify a known colloquial or scientific word in-between four non-words. The 37 items are arranged by increasing difficulty.

Verbal Fluency Test

The Regensburger Wortflüssigkeitstest (RWT) (57) assesses verbal fluency. Specifically, we measured semantic fluency by counting the number of animals and the number of - alternating – fruits and sports, as well as lexical fluency of words starting with the letter "S" and, again alternating, words starting with "G" and "R." For each condition, the participant was given 2 min time.

Auditory Verbal Learning Test

The Verbaler Lern- und Merkfähigkeitstest (VLMT) (58, 59) is a German version of Rey's (60) Auditory Verbal Learning Test (61). The VLMT consists of fifteen words, which are spoken by the examiner on five successive trials. Following each trial, the individual recalls all memorized words. After the five trials, a second group of words (distraction) is read aloud and recalled by the individual. After the distraction list, the individual recalls the words from list one. Following approximately 20 to 30 min, the individual again recalls all words from list one. Finally, the individual is instructed to recognize words from list one from a list of forty-five words. The test measures verbal learning ability, verbal memory consolidation and verbal recognition.

Sentence Reading Test

The sentence reading test, developed by Bergmann and Wimmer (62) requests participants to read short sentences and judge their semantic content. The sentences are of simple content so that erroneous markings are infrequent and the number of sentences marked within 3 min largely reflects reading speed and semantic comprehension.

fMRI Paradigm

Lexical Decision Task (LDT)

When reading single words in the fMRI-scanner, participants completed a lexical decision task (LDT) (63) in which subjects were shown German nouns (targets; e.g., Henker [hangman]) or German non-words (non-targets; e.g. Luicke). Participants had to decide whether the presented stimulus was a correctly spelled German word or not. Non-words were created changing two letters of an existing German noun. The LDT is the most used task to measure lexical access to the visual word form (63–66).

Stimuli

Following the design of Briesemeister et al. (10, 11), we used 120 German four- to eight-letter nouns and 60 non-words in a

2 (discrete emotion) x 2 (affective dimension) within subjects design with 30 items per cell. Contrary to Briesemeister et al. who used happiness and positivity, we tried to extend previous results by using words reflecting the emotion fear and words reflecting the affective dimension of negativity. Stimuli were chosen from the Berlin Affective Word List-Reloaded (67) (BAWL-R) and its discrete-emotion extension, the Discrete Emotion Norms for Nouns BAWL (68) (DENN-BAWL). The BAWL-R is a rating-based word list with norms for affective valence (7-point Likert scale, ranging from negative [-3] to positive [3]) and arousal (5-point Likert scale, ranging from low [1] to high arousing [5]). Given that negativity judgments and BAWL-R's valence ratings are highly correlated (69), words with BAWL-R scores between −0.7 and 0.7 were defined as being of neutral valence (NEU), and words with valence scores below 1 were defined as being of negative valence (NEG). The DENN-BAWL norms were used to classify words as being either strongly or not strongly related to fear, with low-fear words (lowFEAR) having DENN-BAWL scores below 2.0 and high-FEAR words (highFEAR) having scores above 2.0. DENN-BAWL norms indicate the extent to which a single word is related to one of five discrete-emotion categories, with high scores indicating a strong relation. The resulting four orthogonal conditions (lowFEAR + NEU: e.g., "AGENT," Engl. "AGENT"; lowFEAR + NEG: e.g., "MIETE," ENGL "RENT"; highFEAR + NEU: e.g., "MESSER," ENGL. "KNIFE"; highFEAR + NEG "TEUFEL," ENGL. "DEVIL") were uncorrelated for fear and valence scores, indicating that lowFEAR + NEG words are perceived as being negative but not relate to the discrete emotion fear, and vice versa. For statistical details about the stimulus set, see **Table 1**. Mean levels of arousal, imageability, orthographic neighborhood size, frequency of orthographic neighbors, frequency of higher-frequent orthographic neighbors, and the mean number of letters, syllables, bigram frequency, and higher-frequency orthographic neighbors were controlled using analyses of variance (ANOVAs, all *F*s >1; see **Table 1**). The psycholinguistic variables were matched for the highFEAR-versus-lowFEAR and NEU-versus-NEG contrasts and tested with pairwise *t*-tests (all *t*s <1). A list containing all 120 words and 60 non-words is provided in the **Supplementary Materials**. In addition, 30 null-events, serving as baseline, in the form of a fixation cross ("+") were included, which was meant to increase the signal-to-noise ratio of the fMRI paradigm.

fMRI Procedure

Before entering the scanner, participants received oral and written instructions to decide as quickly and accurately as possible via button press whether the presented letter string was a correct German word (left button) or a non-word (right button) of a button box. They were instructed not to press any button when presented with fillers. Eighteen practice trials (twelve words, six non-words, three null-events) that were not part of the stimulus set were used to familiarize the participants with the task. The stimuli were presented in an event-related design on a 32-inch LCD screen, specifically designed for use in an MRI environment (70). The screen resolution was 1,920 × 1,080 pixels, with a screen size of 69.8 × 39.3 cm, a refresh rate of 120 Hz, and a built-in linear luminance look-up table. The display was positioned at the far end of the bore and was viewed via a mirror positioned in the head coil of the MRI scanner. The total viewing distance was 220 cm. The total visible vertical extent of the screen subtended 10.2 degrees visual angle (deg). All stimuli were generated in MATLAB R2013a (Mathworks Inc., Natick, MA, USA) (71) using the Psychophysics toolbox (72). Stimuli were presented using Presentation software (73), which also recorded response times and accuracy of responses. Each trial began with the presentation of a fixation cross (+) in the center of the screen, which was presented for 2,148 ms on average (jitter: 1,401–4,098 ms), followed by the stimulus (800 ms) at the exact same position. The presentation of the words was randomized. The words were presented in black uppercase letters (Arial, font size 50) on a white background. Responses were given through a button box held in the right hand, the right button (blue) for a correct German word and the left button (green) for a non-word. An external pulse from the scanner controlled the start of the first trial.

fMRI Data Acquisition

fMRI data were acquired with a Siemens Magnetom Prisma-fit 3T MRI scanner with a 64-channel head/neck coil. The fMRI run for the lexical decision task consisted of 620 images and lasted about 10 min, including six dummy scans at the beginning. For distortion correction of the functional images an EPI fieldmap sequence based on the Siemens product fieldmapping sequence (TR 623 ms, TE1 4.92 ms, TE2 7.38 ms) was acquired. Structural imaging included a high-resolution T1w (TR 2400 ms, TE 2.24 ms) and T2w sequence (TR 3200 ms, TE 56 ms,), both with 0.8 mm isotropic resolution with sequences from the Human Connectome Project (HCP) Lifespan protocol (74). For preprocessing and statistical analysis, SPM12 software (75), running in a MATLAB R2013a environment (Mathworks Inc., Natick, MA, USA), and additional functions from AFNI (76) were used. Functional images were realigned, de-spiked (with the AFNI 3ddespike function), unwarped, and corrected for geometric distortions using the fieldmap of each participant and slice time corrected. The high resolution structural T1-weighted image of each participant was processed and normalized with the CAT12 toolbox (77) using default settings, each structural image was segmented into gray matter, white matter and CSF and denoised, then each image was warped into MNI space by registering it to the DARTEL template provided by the CAT12 toolbox via the high-dimensional DARTEL (60) registration algorithm. Based on these steps, a skull stripped version of each image in native space was created. To normalize functional images into MNI space, the functional images were co-registered to the skull stripped structural image and the parameters from the DARTEL registration were used to warp the functional images, which were resampled to 3 × 3 × 3 mm voxels and smoothed with a 6 mm FWHM Gaussian kernel. Statistical analysis was performed with a general linear model (GLM) two-staged mixed effects model. In the subject-specific first level model, each condition (discrete emotion: FEAR high/low, emotions dimension: Negativity yes/no) was modeled by convolving stick functions at its onsets with SPM12's canonical hemodynamic response function. Parameter estimates for each condition were

TABLE 1 | Descriptive statistics of the stimulus set, along with the behavioral responses.

	lowFEAR + NEU	lowFEAR + NEG	highFEAR + NEU	highFEAR + NEG	*F*-value	*p*-value
Fear	1.5 (0.26)	1.5 (0.24)	2.2 (0.29)	2.4 (0.34)	84.232	<0.001
Negativity	−0.02 (0.37)	−1.3 (0.26)	−0.05 (0.42)	−1.4 (0.34)	133.389	<0.001
Letters	6.0 (0.69)	6.3 (1.15)	6.0 (0.89)	6.0 (0.93)	0.576	0.632
Syllables	2.1 (0.36)	1.9 (0.67)	2.0 (0.37)	2.1 (0.37)	0.352	0.788
Arousal	3.1 (0.33)	3.0 (0.38)	3.1 (0.55)	3.1 (0.28)	0.793	0.500
Imageability	4.2 (0.96)	3.8 (1.51)	4.3 (1.21)	4.1 (1.24)	0.899	0.444
Bigram frequency	5265.97 (6442.04)	4419.183 (3848.24)	6273.18 (7628.60)	5381.85 (6407.67)	0.444	0.722
Ortho. Neighbors (*N*)	1.2 (1.09)	1.0 (1.59)	1.2 (1.10)	1.0 (1.31)	0.174	0.914
Frequency of *N* (FN)	19.1 (66.56)	38.3 (75.53)	62.6 (171.61)	29.6 (118.11)	0.771	0.513
Higher frequent *N* (HN)	0.3 (0.46)	0.4 (0.77)	0.5 (0.73)	0.6 (0.97)	0.635	0.594
Frequency of HN (FHN)	17.6 (65.79)	36.5 (73.90)	61.1 (171.38)	28.0 (118.31)	0.777	0.509
Frequency	15.7 (17.66)	17.1 (13.81)	12.1 (11.37)	13.8 (19.28)	0.554	0.646
Response times (RT)						
Total	750 (160)	742 (175)	727 (161)	739 (168)		
Control	752 (161)	748 (177)	730 (167)	750 (178)		
Patient	746 (159)	735 (173)	724 (155)	725 (154)		
Error rate (ERR)						
Total	4.3 (6.1)	4.4 (6.2)	3.3 (6.0)	5.5 (6.7)		
Control	3.1 (4.6)	3.3 (4.1)	2.1 (4.6)	4.1 (4.8)		
Patient	5.8 (7.4)	5.7 (8.1)	4.9 (7.2)	7.3 (8.3)		
Response times (RT)	lowFEAR	highFEAR	NEU	NEG	All Conditions	
Total	747 (168)	734 (165)	739 (161)	741 (172)	740 (166)	
Control	751 (169)	741 (173)	742 (164)	750 (178)	746 (171)	
Patient	741 (166)	725 (154)	735 (157)	730 (164)	733 (160)	
Error rate (ERR)						
Total	4.36 (6.17)	4.43 (6.47)	3.83 (6.08)	4.97 (6.51)	4.40 (6.31)	
Control	3.25 (4.37)	3.12 (4.82)	2.61 (4.63)	3.76 (4.50)	3.19 (4.59)	
Patient	5.81 (7.73)	6.17 (7.85)	5.43 (7.30)	6.56 (8.22)	5.99 (7.77)	

calculated via these first-level GLMs, using a temporal high-pass filter (cutoff 128 s) to remove low-frequency drifts and modeling temporal autocorrelation across scans with an AR (1) process (78). For voxel-based group analyses, contrast images for effects of interest were calculated at the first level. These contrast images were used in second level analyses for a words vs. baseline contrast. All results from whole brain analyses are reported at a voxel-level threshold of $p < 0.001$ (uncorrected) with a FWE cluster-level correction of $p < 0.05$.

ROI Analysis

We performed three region of interest (ROI) analyses. The functionally ROI analysis was performed for our a priori regions (i.e., right amygdala, left and right inferior frontal gyrus) using the coordinates used by Briesemeister et al. (11). The amygdala and inferior frontal gyri were selected based on their suggested role in Panksepp's hierarchical emotion theory, representing secondary and tertiary level processes, respectively. The ROIs were created with a sphere of 6 mm in the right amygdala (x = 21, y = 2, z = −11) and in the left (x = −45, y = 35, z = 26) and right inferior frontal gyrus (x = 42, y = 26, z = −8). ROIs were built with the MarsBar toolbox implemented in SPM12. ROI extraction was performed with REX (79), based on the average contrast estimates of our four word conditions, for further statistical analysis.

Statistical Methods

Statistical analyses were performed in SPSS (version 18) (80) and R (version 4.0.5) (81). First, we defined primary, secondary and tertiary outcomes. Primary outcomes were any effects of activation in a 2 (group: JMEs/HCs) x 2 (discrete emotion: FEAR high/low) x 2 (emotions dimension: Negativity: yes/no) repeated measures ANOVA of our fMRI-data. Secondary outcomes were behavioral responses in the fMRI-paradigm (response times and error rates), again tested in a 2x2x2 design using a non-parametric ANOVA type test for repeated measure designs provided by the R package nparLD (82). *Post-hoc* contrasts in differences for the relative treatment effect were computed using a normal approximation with a Fisher transformation and the delta method, as described in Gunawardana and Konietschke (83). Mean lexical decision response times (LDRTs) were calculated for each participant and condition after the exclusion of non-responders, behavioral errors, and outliers, which were defined as responses faster than 300 ms or slower than 1,500 ms. In total 3.38 and 3.19% of responses were

TABLE 2 | Descriptive statistics of sample.

	Control group ($n = 62$)				JME patients ($n = 47$)			
	***n* (%)**	**Min**	**Max**	**Mean (SD)**	***n* (%)**	**Min**	**Max**	**Mean (SD)**
Sex								
Female	33 (53.2%)				24 (51.1%)			
Male	29 (46.8%)				23 (48.9%)			
Age	62	18	62	27.71 (9.69)	47	14	52	27.09 (7.84)
Education in years	62	10	18	13.18 (1.94)	47	8	17	12.28 (2.10)
Epilepsy begin (age)				47	3	24	14.23 (3.83)	
Epilepsy duration (in years)				47	0	34	12.85 (7.70)	
Seizure type (mulitple types in one patient)								
GCTS				15 (31.9%)				
Absences				12 (25.5%)				
Myoclonus				29 (61.7%)				
Seizure free				16 (34.0%)				
Medication								
No Medication					3 (6.2%)			
Monotherapy					28 (58.3%)			
Polytherapy					17 (35.4%)			
Medication (multiple medications in one patient)								
Levetiracetam					34 (72.3%)			
Valproic acid					12 (25.5%)			
Lamotrigine					7 (14.9%)			
Zonisamide					2 (4.3%)			
Topiramate					1 (2.1%)			
Other					7 (14.9%)			
Any antidepressant 0 (0.0%)					5 (10.6%)			
Psychiatric disorder (SCID 1&2)								
No PD	49 (80.3%)				18 (39.1%)			
with PD	12 (19.7%)				28 (60.9%)			

Shown are absolute numbers and percentages. PD, psychiatric disorders; SCID, Structured Clinical Interview for DSM-IV Disorders. One psychiatric diagnosis (SCID) missing in control and patient group.

filtered for healthy controls and patients with JME, respectively. Error rates (ERRs) were calculated as the summed errors per condition and participant. Additionally, neuropsychological measures were defined as secondary outcomes. Group differences in neuropsychology variables were computed with univariate comparisons between groups, done via a non-parametric *t*-test (Brunner-Munzel Test) using the R package rankFD (84). Tertiary outcomes used the same model for behavioral responses as in the secondary outcomes but with an additional between subject factor for psychiatric comorbidities and the same model computed for patients only with type of seizure as a between subject factor (generalized tonic clonic seizures vs. absences and myoclonic seizures only vs. seizurefree). Further, we repeated the analysis of the secondary outcomes replacing group with type of antiseizure medication (mono- vs. polytherapy) only for patients with JME. We also conducted a repeated measures ANCOVA with 2 (discrete) x 2 (dimension) as within subject factors and duration of epilepsy as covariable only in patients, using the R-package nlme (85). In order to maintain control of the type I error and yet ensure adequate statistical power we employed the following scheme for *p*-value adjustment in our neuropsychological comparisons. For the primary outcomes, we used the Bonferroni procedure to control the FWER for each spherical regions of interest (i.e., multiplying the *p*-values by three). For secondary outcomes we used the Benjamini-Yekutieli (86) procedure to control the FDR at a level of 0.05. For tertiary outcomes, we did not conduct any correction for multiplicity as we considered them to be auxiliary analyses. For *p*-values we provide the unadjusted and adjusted version. Confidence intervals are unadjusted.

The effect measure used for primary outcomes was $\eta 2$ and for non-parametric analyses (secondary outcomes) the *RTE*. For comparisons between two groups *RTE* is the probability that a random subject from one group has a higher value in the outcome variable than a random subject from the other group. It is identical to the area under the curve when using the outcome variable to classify subjects into the two groups. For more than two groups it is the probability that a random subject from one group has a higher value in the outcome variable than a random subject from the total sample.

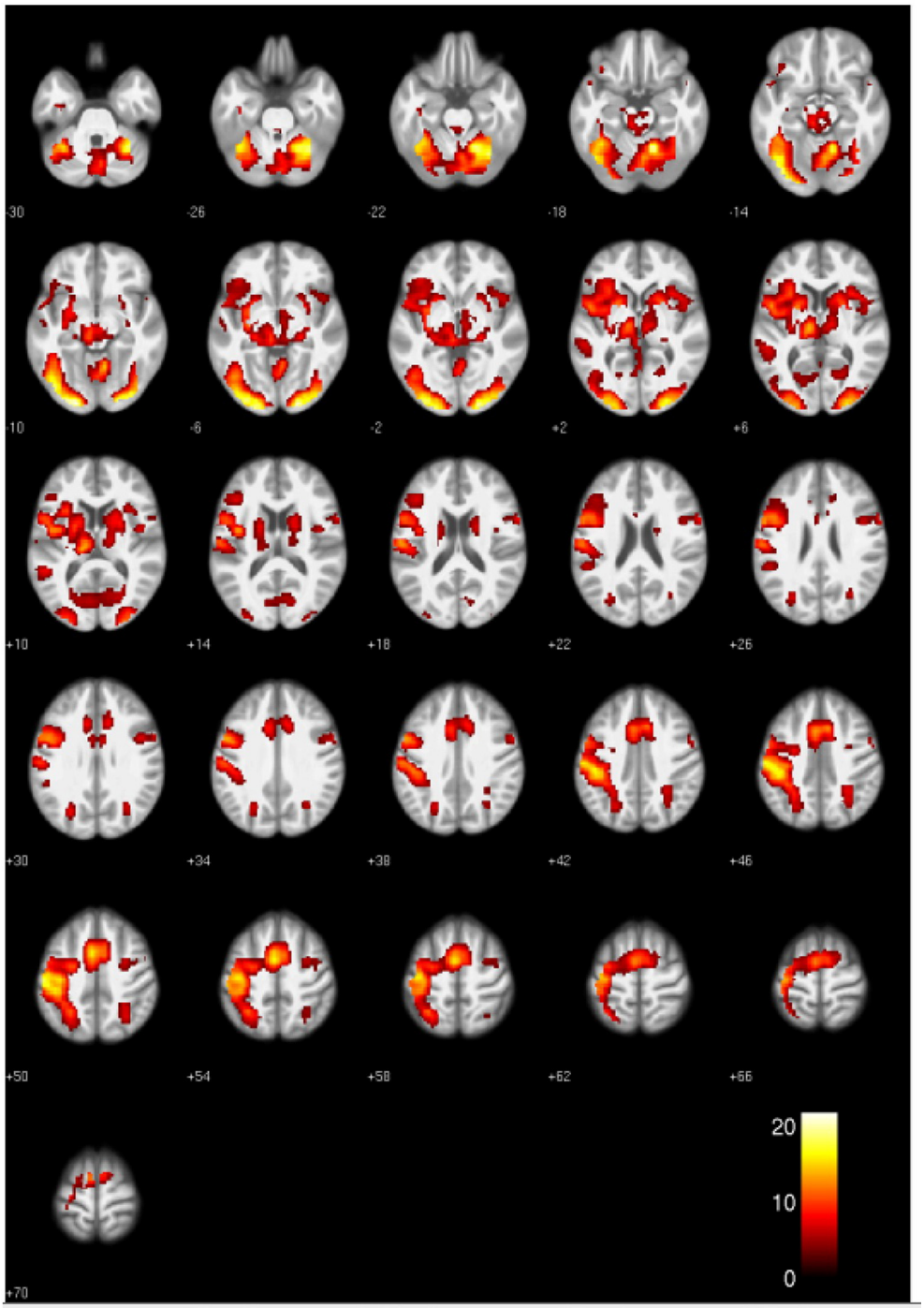

FIGURE 1 | Activation clusters revealed by the whole brain analyses. Regions that elicited increased activation for words compared to baseline (irrespective of group) are shown in yellow. All clusters were extracted at a threshold of $p < 0.001$ [uncorrected, with a FWE cluster-level-correction ($p < 0.05$)].

RESULTS

Clinical Features

Twenty-eight patients (60.9%) and 12 controls (19.7%) presented a psychiatric disorder. 15.2% of patients had Axis I, 23.9% Axis II and 21.7% Axis I & II disorders (see **Table 2**). Considering multiple diagnoses in one patient, affective (15.2%), anxiety (13.0%), substance-related disorders (13.0%) and obsessive-compulsive personality disorders (OCD; 21.7%) represented the majority of psychiatric comorbidities. For a detailed description of psychiatric diagnoses see **Supplementary Table 1**. Due to multiple sessions, incompliance and tiredness of study

participants, not all neuropsychological subtests have been performed in all patients (**Supplementary Table 2**).

Neuropsychology

In our non-parametric ANOVA type test (82) patients had significantly lower scores in the verbal intelligence test than healthy controls ($p = 0.01$). Patients with JME performed worse in single phonematic fluency ($p = 0.01$) as well as single ($p = 0.06$) and alternating semantic fluency ($p < 0.001$). Reading speed was decreased in patients with JME compared to healthy controls ($p < 0.001$). Despite having significantly lower scores than healthy controls, patients performed, for the most part, on average in all subtests. There were no differences in verbal memory functions: total learning score, delayed recall and recognition. All reported results were significant after correction for multiple comparisons. For a detailed description see **Supplementary Table 2**.

Behavioral Results: Lexical Decision Response Times and Error Rates

A 2 (group: JMEs/HCs) x2 (discrete emotion: FEAR high/low) x2 (emotion dimension: Negativity: yes/no) non-parametric repeated measures ANOVA revealed a significant main effect of fear ($p = 0.006$) that was driven by faster responses of highFEAR words compared to lowFEAR words (detailed descriptive statistics are presented in **Table 1**, inferential statistics in **Table 4**, and in **Supplementary Figure 1**). Moreover, a FEAR x Negativity interaction approached significance ($p < 0.001$). Planned pairwise comparisons between all conditions revealed faster response times of highFEAR+NEU compared to lowFEAR+NEU words ($p < 0.001$). Error rates showed a significant main effect of group with healthy controls making significantly less errors ($p < 0.001$). A significant main effect of Negativity ($p < 0.001$) that was driven by an increased number of errors in response to words of negative valence (NEG) compared to words with neutral valence (NEU). Further, a FEAR x Negativity interaction ($p < 0.001$) was driven by more errors for highFEAR+NEG than highFEAR+NEU words. *Post-hoc* sensitivity analysis adding psychiatric comorbidity or type of seizure (generalized tonic-clonic seizures x myoclonus and/or absences x seizure free) did not change the significance of the aforementioned effects regarding response times and error rates. Further, no group or interaction with group was significant when comparing antiseizure medication (mono- vs. polytherapy) in patients only. Duration of epilepsy did not correlate with either, response time or error rate in the repeated measures ANCOVA.

Neuroimaging Results

Whole Brain Analysis

The voxel-based whole-brain analysis across both groups revealed a main effect for words compared to fixation-baseline. Higher activity for words was found in a number of regions commonly involved in word processing, including an extended cluster in the left and right occipital lobe including inferior occipitotemporal, parietal and frontal areas, a left medial temporal cluster, a right occipitoparietal cluster, a right parahippocampal cluster and a cluster located in the brainstem. Please see **Figure 1** and **Table 3** for details. We did not observe any statistically significant differences between patients with JME and controls in the words vs. fixation-baseline contrast.

TABLE 3 | Significant cluster of the whole brain analysis.

	MNI coordinates			Volume (voxels)	
Region	***x***	***y***	***z***		***T***
Stimulus main effect					
Words > baseline					
L middle temporal	−50	−44	10	196	7.40
R parietooccipital	28	−47	48	323	7.30
R parahippocampal	28	−2	50	129	6.23
Brainstem	0	−37	−38	70	5.52
L/R posterior occipital, occipitotemporal, parietal, frontal				14,851	
L occipitial	−20	−92	−10		21.50
R occipital	20	−92	−8		21.06
R inferior occipitotemporal	16	−92	−20		20.11
R fusiform gyrus	38	−54	−15		9.93
L primary somatosensory	−50	−20	48		18.05
L primary motor	−40	−4	12		14.21
R insula	33	18	5		8.84
R putamen	23	10	2		10.52
L pars opercularis	−54	6	25		12.49
R pars opercularis	33	18	5		7.64

Data were extracted at a voxel-level threshold of p < 0.001 (uncorrected) and a cluster-level threshold (FWE) of p < 0.05.

ROI Analyses

A 2 (group: JMEs/HCs) x 2 (discrete emotion: FEAR high/low) x 2 (affective dimension: Negativity yes/no) repeated measures ANOVA revealed a main effect of affective dimension in the right inferior frontal gyrus ($p = 0.001$), which was driven by higher activity in response to words with negative valence compared to words with neutral valence. The same repeated measures ANOVA in the right amygdala revealed a significant interaction of fear and negativity ($p = 0.018$), driven by higher activity in response to words with high fear values and negative valence compared to words with low fear values and negative valence. No differences were found in the left inferior frontal gyrus. A main effect of fear in the right amygdala ($p = 0.06$) driven by higher activity for words with high fear compared to low fear, as well as a main effect of group in the right inferior frontal gyrus ($p = 0.06$), driven by higher activity in patients with JME compared to healthy controls, did not reach significance after Bonferroni *p*-value correction. Please see **Table 4** and **Figure 2** for details.

TABLE 4 | Statistical inference for the behavioral and fMRI data.

Effect							χ^2 **- value**	***p* – value adjusted**	***RTE* difference**
LDRT									
Main Effect of FEAR						10.53	0.006		
FEAR x Negativity Interaction					12.42	<0.001			
lowFEAR + NEU > highFEAR + NEU					16.47	<0.001	0.047		
lowFEAR + NEG < higFEAR + NEG					0.04	1	0.001		
ERR									
Main effect of group						13.06	<0.001		
JME > HC							12.75	<0.001	0.135
Main effect negativity						15.63	<0.001		
FEAR x Negativity Interaction					11.64	<0.001			
lowFEAR + NEG > lowFEAR + NEU					0.10	1	0.009		
highFEAR + NEG > highFEAR + NEU					30.31	<0.001	0.151		
				MNI coordinates					
Anatomical location (spherical ROI)	L/R	BA	*x*	*y*	*z*	Size	*F* - value	*p* – value adjusted	η^2 - value
Main effect of negativity									
Inferior frontal gyrus	R	47	42	26	−8	6 mm	13.46	0.001	0.112
Inferior frontal gyrus	L		−45	35	10	6 mm	2.10	0.450	0.019
Amygdala	R		21	2	−11	6 mm	1.90	0.513	0.017
Main Effect of FEAR									
Amygdala		R		21	2	−11	6 mm	5.59	0.060	0.050
FEAR x Negativity Interaction									
Amygdala		R		21	2	−11	6 mm	7.73	0.018	0.067
Main effect of group									
Inferior frontal gyrus		R	47	42	26	−8	6 mm	5.54	0.060	0.049
Inferior frontal gyrus		L		−45	35	10	6 mm	0.89	1	0.008
Amygdala		R		21	2	−11	6 mm	0.08	1	0.001

Anatomical locations for selected main effects and interactions of negativity, fear and group. Anatomical p-values are adjusted for number of ROIs (N = 3). LDRT, response time in lexical decision task; ERR, error rate (in %) in lexical decision task; LDRTs and ERRs p-values are adjusted with the Benjamini-Yekutieli method, together with all other secondary variables.

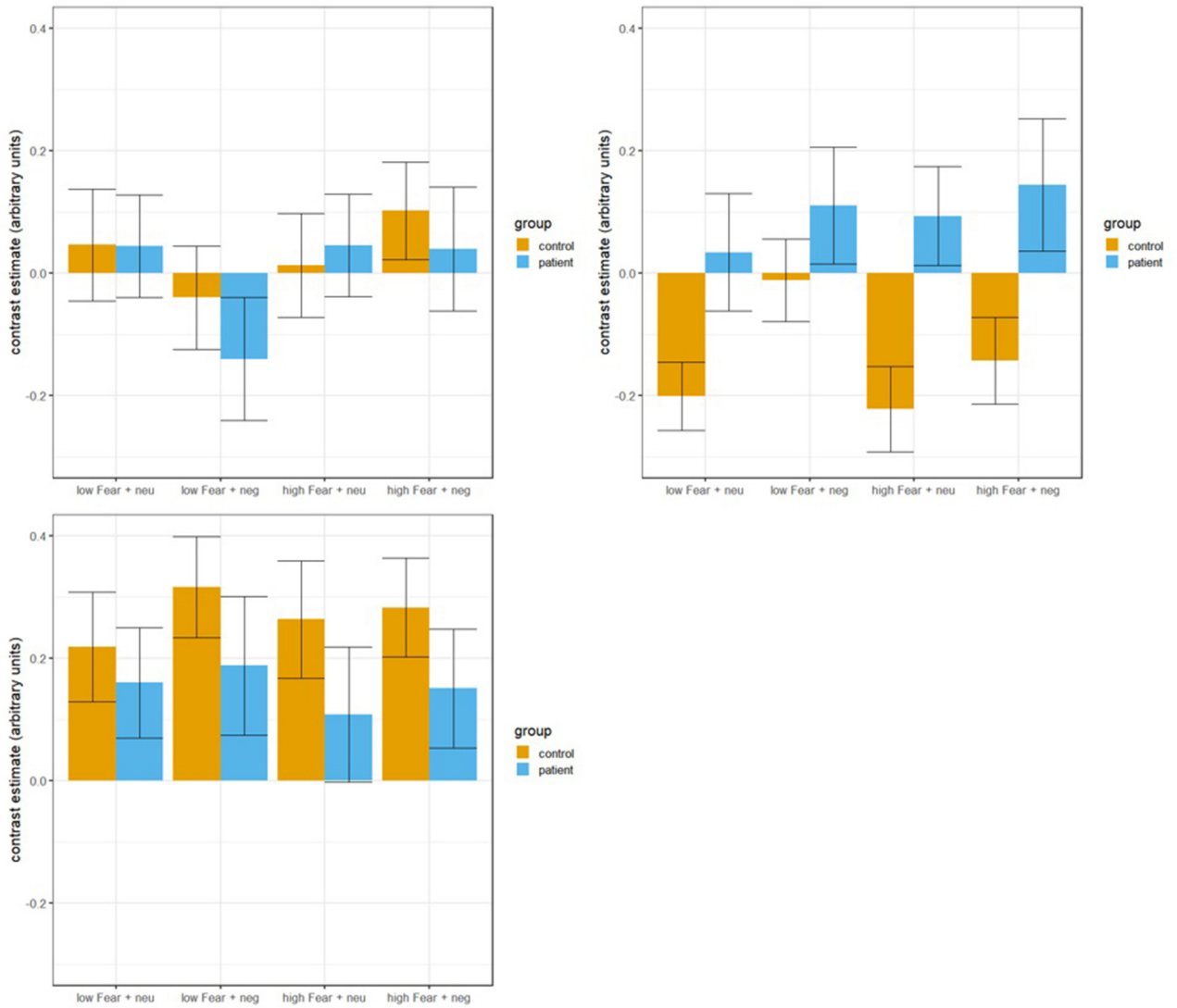

FIGURE 2 | fMRI results for our conditions in the right amygdala (top left), right inferior frontal cortex (top right), and left inferior frontal gyrus (bottom left). Errror bars indicate standard error.

DISCUSSION

With this study we aimed to validate and to extend previous findings suggesting hierarchical processing of emotion (10, 11, 13). In support of our hypothesis, we found an interaction of discrete emotion and affective dimensional processing in the right amygdala, likely to reflect secondary level processing. Further, brain activation in the right inferior frontal gyrus points to affective dimensional processing, suggested to reflect tertiary level processing. The processing of emotional words was not influenced by psychiatric conditions, type of seizure or duration of epilepsy, as well as mono- vs. polytherapy in JMEs only. Patients and healthy controls did not show any differences in brain activity or response times, despite higher incidences of psychiatric conditions and slightly decreased verbal intelligence, verbal fluency and reading speed in patients with JME.

Based on the results of Briesemeister et al. (10, 11) and the idea that affectively conditioned stimuli like words access secondary and tertiary process levels within Pankespp's hierarchical emotion model (13), we expected the amygdala and the inferior frontal gyrus, to be involved in implicit emotional word processing (19, 22, 23, 87). Therefore, we tested healthy controls and patients with JME which are suggested to have cognitive, behavioral and emotional disturbances, related to subtle structural and functional alterations in frontal brain regions (44–47). Furthermore, we were interested in verbal and neuropsychological functions of patients with JME, and if psychiatric conditions, type of seizure, mono- vs. polytherapy, and duration of epilepsy were related to discrete

emotion and affective dimensional processing. The whole brain analysis revealed brain regions typically activated in reading emotional words (22), involving occipital, temporal, parietal and frontal regions. Activity in the right amygdala in response to the emotional content of words, confirmed the results of Briesemeister et al. (11) and Nakic et al. (19), supporting the idea of an amygdala involvement in implicit emotion processing. According to Nakic et al. (19), amygdala activity indicates the processing of emotional salience, and regions relevant for behavioral responses such as the medial orbitofrontal gyrus and the anterior cingulate cortex receive input from the amygdala, thereby facilitating behavioral lexical decision responses. In support of this proposal, we observed an interaction of discrete emotion and affective dimension driven by higher activity in response to high fear words with negative valence compared to low fear words with negative valence in the right amygdala. In line with this pattern of activity, response times revealed a significant main effect of fear, driven by faster response times for high fear words compared to low fear words, and an interaction of discrete emotion and affective dimensional processing, driven by faster response times for low fear words with negative valence compared to low fear words with neutral valence. These behavioral interactions support Nakic et al. (19) findings, of a correlation between amygdala and anterior cingulate cortex activity in conditions showing enhanced word processing speed for negative words. Activity in the right inferior frontal gyrus revealed a main effect of negativity, driven by higher activity for negative compared to neutral words, which confirms Briesemeister et al's. results, and are line with the suggestion of the hierarchical emotion model of explicit evaluation of emotional salient stimuli on tertiary levels lending further support to Panksepp's proposal, that tertiary level processes "require expansive neocortical tissues that permit linguistic-symbolic transformations" (88). Thus, activity in the inferior frontal gyrus could reflect increased computational demands within the so-called *reading network* underlying semantic evaluation and integration processes of emotional information (20, 89–91).

However, the interaction of discrete emotion and affective dimensional processing in the amygdala in the current study is in contrast with Briesemeister's double dissociation of discrete emotion and affective dimension. Thus, it further challenges the amygdala's role in emotion processing. Briesemeister et al. (10, 11) suggested that activity in the inferior frontal gyrus in response to the affective dimension of positivity and the activity in the amygdala in response to the discrete emotion happiness reflect the dissociation of discrete emotion and affective dimensional processing in the brain. However, as the hierarchical emotion model focuses on primary process level emotions, the amygdala's role – as a potential secondary level process – is not yet clear, and the current results do not necessarily contradict the hierarchical emotion model. The observed activity in the amygdala could be based on the processing of biological relevant information and could reflect the interaction of top-down affective dimensional and bottom-up discrete emotion information carried by conditioned affective stimuli such as words (20, 92, 93).

The absence of significant differences in brain activity and response times between patients with JME and healthy controls during implicit emotion processing could be interpreted in favor of the notion that emotion processing in reading operates rather independently of other cognitive domains, as previously reported in emotional face recognition (94). However, these results were somewhat unexpected, as patients with JME were reported to present structural and functional differences in frontal regions, as well as neuropsychological deficits, related to executive functions (47). These results might be attributable to the implicit nature of the lexical decision paradigm and could point to independent processing of emotional information in reading, despite known altered explicit executive functioning in JME (37, 39, 40, 47). In addition, this result could suggest rather unaffected implicit emotion processing in patients with JME and could emphasize the importance of a differentiation of the implicit and explicit nature of tasks used in studying emotion processing, considering that most tasks used are of explicit nature (25–27, 29, 95–98). Error rates revealed a significant main effect of negativity, driven by higher error rates in response to negative as compared to neutral words, and an interaction, showing higher error rates in response to words with high fear values and with negative valence compared to words with high fear values and neutral valence. Further, we found a statistically significant difference in error rates between healthy controls and patients with JME. However, as the total number of errors was 3.19% (healthy controls) and 5.99% (patients with JME), respectively, we interpret this to be in a normal range and carefully interpret this difference to be due to impaired cognitive functions, as revealed by the results of the neuropsychological testing.

Neuropsychology revealed slower reading-speed, lower semantic and phonematic verbal fluency, and reduced verbal crystalline intelligence in patients with JME. Slower reading speed and comprehension extends on previous results found in patients with temporal lobe epilepsy and idiopathic epilepsies in children (99–101). Deficits in verbal fluency in our patients confirm existing results in patients with JME, pointing to disturbed executive processing supporting epilepsy-specific patterns of neuropsychological dysfunctions (99, 102). Reduced verbal intelligence might be interpreted as a constitute of reading ability and verbal executive functioning, as overall intelligence level is usually normal in this patient group (103, 104). Additionally, we found no significant association between psychiatric conditions, type of seizure disorder or epilepsy duration and the outcome of emotional word processing in the lexical decision task.

This study has some limitations related to the interpretation of neuropsychological data and brain activity in response to the emotional content of words. First, as psychiatric disorders in JME patients are reported to be increased, a special focus on the type of psychiatric disorder and a direct comparison between those may unveil additional information (105). For simplicity, we only controlled statistically for psychiatric disorders (yes or no) within our groups and their effect on affective processing. Second, as argued by Hamann (106), our fMRI-results might reveal deeper insights into brain mechanisms of implicit affective

processing if we were to have presented more types of emotions than only fear/negativity in our paradigm. There are, however, some strengths that we like to highlight. This study validates and replicates the rationale of a previous study (11), using a different set of emotions. Due to our implicit task design and with the inclusion of patients with JME, we were able to unveil unaffected implicit affective processing in this patient group. To our knowledge, this is the first study to report results of implicit affective processing in this patient group, as most studies use paradigms which focus on explicit emotion processing (26, 29, 97, 98) (e.g., emotion recognition).

In this study, we showed an interaction of discrete emotion and affective dimensional processing in the right amygdala. This interaction was driven by higher activity in response to words with high fear values and negative valence, compared to words with low fear values and negative valence. We interpreted this activity to reflect the interaction of top-down and bottom-up circuits of primary and tertiary process levels in the amygdala, which is suggested to be involved in the conditioning of emotional stimuli in general. Higher activity in the right inferior frontal gyrus for negative words is in line with the results of Briesemeister et al. and was interpreted to signal explicit semantic evaluation and emotional memory integration on the tertiary process level. The results of the neuropsychological testing revealed subtle deficits in reading speed, phonematic and semantic fluency, and verbal intelligence in patients with JME compared to healthy controls. However, this did not result in any behavioral differences concerning discrete emotion and affective dimensional processing of patients with JME and healthy controls. Thus, the results of the current study could point to unimpaired implicit emotion processing of patients with JME during lexical decisions to words carrying emotional information.

DATA AVAILABILITY STATEMENT

The datasets presented in this article are not readily available because The decision of the Ethics Committee of the state of Salzburg does not include the sharing of the data. Requests to access the datasets should be directed to LR, lucas.rainer@stud.sbg.ac.at.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Salzburg Ethics Committee. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

LR, JH, GK, and MB contributed significantly to conception and design of the presented paper, acquisition, analysis and interpretation of the data as well as drafting of the paper. ES, PL, LK, SS-Y, MKi, MKr, GZ, and ET contributed to acquisition and analysis of data and revising the paper for intellectual content. GK, JH, MB, and ET contributed significantly to conception of the study, interpretation of the results and gave final approval of the submitted version of the manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2022.875950/full#supplementary-material

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Mesial-Temporal Epileptic Ripples Correlate With Verbal Memory Impairment

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Rationale: High frequency oscillations (HFO; ripples = 80–200, fast ripples 200–500 Hz) are promising epileptic biomarkers in patients with epilepsy. However, especially in temporal epilepsies differentiation of epileptic and physiological HFO activity still remains a challenge. Physiological sleep-spindle-ripple formations are known to play a role in slow-wave-sleep memory consolidation. This study aimed to find out if higher rates of mesial-temporal spindle-ripples correlate with good memory performance in epilepsy patients and if surgical removal of spindle-ripple-generating brain tissue correlates with a decline in memory performance. In contrast, we hypothesized that higher rates of overall ripples or ripples associated with interictal epileptic spikes correlate with poor memory performance.

Methods: Patients with epilepsy implanted with electrodes in mesial-temporal structures, neuropsychological memory testing and subsequent epilepsy surgery were included. Ripples and epileptic spikes were automatically detected in intracranial EEG and sleep-spindles in scalp EEG. The coupling of ripples to spindles was automatically analyzed. Mesial-temporal spindle-ripple rates in the speech-dominant-hemisphere (left in all patients) were correlated with verbal memory test results, whereas ripple rates in the non-speech-dominant hemisphere were correlated with non-verbal memory test performance, using Spearman correlation).

Results: Intracranial EEG and memory test results from 25 patients could be included. All ripple rates were significantly higher in seizure onset zone channels ($p < 0.001$). Patients with pre-surgical verbal memory impairment had significantly higher overall ripple rates in left mesial-temporal channels than patients with intact verbal memory (Mann–Whitney-U-Test: $p = 0.039$). Spearman correlations showed highly significant negative correlations of the pre-surgical verbal memory performance with left mesial-temporal spike associated ripples ($r_S = -0.458$; $p = 0.007$) and overall ripples ($r_S = -0.475$; $p = 0.006$). All three ripple types in right-sided mesial-temporal channels did not correlate with pre-surgical nonverbal memory. No correlation for the difference between post- and pre-surgical memory and pre-surgical spindle-ripple rates was seen in patients with left-sided temporal or mesial-temporal surgery.

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Discussion: This study fails to establish a clear link between memory performance and spindle ripples. This highly suggests that spindle-ripples are only a small portion of physiological ripples contributing to memory performance. More importantly, this study indicates that spindle-ripples do not necessarily compromise the predictive value of ripples in patients with temporal epilepsy. The majority of ripples were clearly linked to areas with poor memory function.

Keywords: epilepsy, temporal lobe epilepsy, high frequency oscillations (HFO), ripples, memory, sleep spindles

INTRODUCTION

Despite optimized medical treatment approximately one-third of all patients with epilepsy continue to suffer from epileptic seizures (1). Among these intractable epilepsies, temporal lobe epilepsy is the most common cause. In total, 70% of cases with mesial-temporal lobe epilepsy (mTLE) are associated with mesial-temporal sclerosis (2, 3). Surgical removal of epileptic tissue is a curative option for refractory epilepsy. In temporal lobe epilepsies, selective amygdalohippocampectomy (sAHE) and anterior temporal lobe resection (ATL) are the main evidence-based surgical options (4). In order to perform successful surgery, epileptologists have to define the seizure onset zone (SOZ) in patients, which is defined as the brain area that generate seizures (5). Non-invasive diagnostics in some patients, such as scalp electroencephalography (EEG) and MRI are inconclusive or too vague to identify the SOZ. In these cases, intracranial EEG (iEEG) can help to define those brain areas generating seizures.

In the last two decades, high frequency oscillations (HFO; ripples = 80–200, fast ripples 200–500 Hz) became promising biomarkers for epileptic activity. An increased occurrence of HFO in the SOZ could be seen in many clinical studies with patients suffering from medically refractory epilepsy (6–9). Some studies showed that HFO were more specific for the SOZ than interictal epileptic spikes (IES) (10, 11). More importantly, many studies and a meta-analysis showed a correlation of the removal of HFO-generating brain tissue with seizure-free outcome, in some cases even superior to the removal of the SOZ or the Irritative Zone (12–14). However, a multicenter trial and other studies indicated that HFO can only predict outcomes in some but not all patients (13, 15–17), and another study recently showed no benefit of HFO as biomarkers in comparison to IES (18).

When using HFO to delineate the epileptic zone one major problem is the coexistence of epileptic and physiological HFO. Physiological HFO, mainly in the ripple band, can be found in all brain regions (19) and cannot be distinguished from epileptic ripples by their frequency characteristics (20, 21). In neocortical regions, physiological ripples are often seen as task-related or induced by sensory stimulation (22–24). In mesial-temporal regions, physiological ripples play an important part in a fine-tuned mechanism of memory consolidation during non-rapid-eye-movement-sleep (25). During up-states of cortical slow waves (<1 Hz), the thalamus is stimulated to produce cortico-thalamical sleep spindles, transient (0.5–2 sec) oscillatory activity between 12 and 16 Hz, seen in frontal and parietal scalp EEG contacts (26). During these sleep spindles, the thalamic "gate" is closed for sensory input, providing ideal conditions for unimpaired memory transfer via synchronous mesial-temporal ripple activity (27–31).

In patients with epilepsy, the distinction between physiological and epileptic activity is extremely challenging, as epileptic and physiological interictal HFO both occur together in time and space (32). However, the co-occurrence of ripples with scalp EEG spindles enhances the chance to extract physiological activity. Furthermore, ripples co-occurring with sleep spindles showed different amplitude properties than epileptic IES-associated ripples (33, 34). More importantly, coupling of ripples to sleep spindle troughs (i.e. the depolarized down-states of sleep spindle oscillations) could be seen (33, 35). These spindle (-trough) coupled ripples especially form a likely reliable subgroup of physiological ripples.

In the present study, we aim to investigate whether a higher rate of spindle-ripples in mesial-temporal regions correlates with memory function. During the pre-surgical investigation, patients undergo neuropsychological evaluations to identify brain regions that show decreased function due to epileptic activity. Specific memory tests can aid to distinguish between left- and/or right-sided impairment of memory function. In a patient with a left-hemispherical speech-dominance the left temporal lobe including the hippocampus is usually linked to verbal memory function (36), whereas the right hippocampus is linked to non-verbal memory function such as the memory of faces, figures, and shapes (37).

The results of these detailed pre-surgical investigations can be correlated with the amount of HFO visible in the mesial-temporal structures during an iEEG investigation. If we are able to identify a subgroup of HFO (like spindle-ripples) in the hippocampus that is clearly linked to memory function this would allow two crucial advances in HFO research. First, we would improve the differentiation between physiological and epileptic HFO. Second, a subgroup of HFO could be used as a neurophysiological measure of memory function beyond neuropsychological testing.

For this study, we used a unique dataset of patients who underwent iEEG and received neuropsychological testing before and after epilepsy surgery. Regarding ripple rates in mesial-temporal structures of patients with suspected epileptic mesial-temporal lobe involvement, we hypothesize that spindle coupled ripples correlate with pre-surgical memory performance. In consequence, we hypothesize that surgical removal of

spindle-ripple-generating tissue results in a deterioration of memory performance. In contrast to spindle-ripples, we hypothesize that the majority of mesial-temporal ripples and IES-associated ripples are epileptic and each correlate with impairment of memory function.

METHODS

Patient Selection

All patients that underwent chronic intracranial EEG (iEEG) monitoring at Freiburg Epilepsy Center between January 2012 and July 2019 were considered for inclusion. Intracranial EEG was only performed for clinical reasons. Electrode locations were solely selected according to clinical needs and designed in an interdisciplinary surgical conference according to seizure semiology, scalp- EEG, neuroimaging, and neuropsychological evaluation. EEG evaluation and the SOZ determination were performed by experienced neurophysiologists independent of this study. All patients gave written informed consent to the EEG analysis for scientific purposes and the Ethics Committee of the Freiburg University Medical Center agreed to the study.

The inclusion criteria for this study were as follows:

- Unilateral or bilateral depth electrode implantation in mesial-temporal structures (hippocampus and/or parahippocampal gyrus).
- Simultaneous intracranial and scalp EEG recordings.
- EEG sampling rate of at least 2,000 Hz.
- Epilepsy surgery after the iEEG investigation.
- Successfully completed pre- and post-surgical memory assessment at the Freiburg Epilepsy Center.

Recording Methods

Intracranial depth electrodes were implanted. Electrodes were made of Platinum/Iridium (Dixi Medical, Besancon, France) with five to 18 contacts and a diameter of 0.8 mm. A digital video system called "Profusion EEG Software" (Compumedics Limited, Abbotsford Victoria, Australia) was used to record iEEG. The sampling rate was 2,000 Hz using a digital low-pass filter with a cutoff frequency of 800 Hz. Scalp EEG electrodes were placed according to the international 10–20-system. Electrooculogram and electromyogram were added on the second day after the intracranial electrode implantation. Sleep staging was performed according to the American Academy of Sleep Medicine guidelines by trained technologists.

EEG Selection

Sleep spindles and ripples are predominantly prevalent in the N2 stage of slow wave sleep (38, 39) therefore only N2 stage periods were chosen. For each patient 30 min of iEEG with a simultaneous scalp EEG was selected. Only periods with at least 1 h distance to subclinical or clinical seizure were considered.

EEG data were converted into binary format and high-pass-filtered using the "ASA" software" (ANT Neuro, Enschede, Netherlands) via 2nd Butterworth filter with a cut-off-frequency of 0.5 Hz. After that, EEG files were transformed into the edf-format. The first 5 min of every EEG were screened by a neurophysiologist and contacts with continuous artifacts in intracranial or scalp EEG were rejected from further analysis.

Automatic Detection

HFO and IES were automatically detected in all hippocampal and parahippocampal (afterwards stated as mesial-temporal) iEEG channels. Frontal and parietal sleep spindles were detected on the simultaneous scalp EEG. For both analyses, a previously published detector was used (40). This detector is based on the multivariate classification of iEEG epochs using kernelized support-vector-machines.

The ripples used to train the detectors were visually marked by experts. Ripples are oscillations in the iEEG that clearly stand out in amplitude from the basic iEEG activity and consist of at least four consecutive oscillations. IES are fast-transients common to epileptic patients, that are generated by a hyper-excitability of neural networks and have durations shorter than 250 ms. A distinct set of features was calculated for ripples and IES. These features served to characterize iEEG segments/epochs with a duration of 25 ms and 50% overlap with the adjacent epochs, thus providing a 12.5 ms time resolution. The iEEG epochs represented the inputs fed to the support vector machine (SVM classifiers). The classified epochs then formed the ripple and IES detections based on the following rules:

- All epochs generating an output higher or equal to the ripple and IES specific threshold were classified as positive epochs.
- Ripple and IES detections were formed by those immediately adjacent and positive epochs.
- Ripple and IES detections having durations longer than 250 ms were eliminated.
- Detections from the same ripple and IES detections and less than 25 ms apart from each other were joined, if the duration of the joined detection exceeded 250 ms, the lowest start time was kept and a duration of 250 ms was defined.

IES-Ripple consisted of, respectively, ripple detections that originated from the same channel as IES detection and had at least a 75% temporal coincidence with the same IES detection.

The detected sleep spindles were scalp EEG events with an oscillatory waveform, a duration between 0.5 and 3.0 s, and an oscillating frequency between 11 and 16 Hz. The detection was firstly based on the calculation of a set of features that characterized the EEG trace. Each feature was calculated for scalp EEG segments/epochs with a duration of 100 ms. These characterized EEG epochs were the events fed to and classified by the SVM. Once the classification of the scalp EEG segments was done, the sleep-spindle detections were then formed based on the following rules:

- All epochs generating an output higher or equal to the SVM threshold were classified as positive epochs.
- Spindle detections were formed by all of the immediately adjacent positive epochs.
- Spindle detections with a duration shorter than 500 ms were eliminated.
- Spindle detections having a duration longer than 3.0 s were eliminated.

- Spindle detections less than 500 ms apart from each other were joined, if the duration of the joined detection exceeded 3.0s, the lowest start time was kept and a duration of 3.0 s was defined.

The features used for the multivariate classification described the amplitude, waveform, and frequency characteristics [see also (34, 41)] and were also based on the raw, filtered, and wavelet-transformed signals. The description of the feature calculation and selection are described in the corresponding publications, as well as the procedures followed for the training, validation, and testing of the detectors. A custom MATLAB 2018b script was used to determine whether ripples were co-occurring with sleep spindles and IES. Previously published methods from our group were used to determine whether ripples were coupled to spindle troughs (33, 42).

Figure 1A shows an example of an EEG sleep spindle with co-occurring iEEG ripples. **Figure 1B** shows examples iEEG IES with iEEG ripples.

Neuropsychological Evaluation

Verbal memory performance was evaluated using the Verbaler Lern- und Merkfaehigkeitstest (VLMT) (43), a modified German version of the Rey Auditory Verbal Learning Test – RAVLT (44). The VLMT tests serial learning and immediate recall of 15 words in five consecutive learning trials, free recall after distraction as well as delayed free recall and recognition of the target words after a 30-min delay. Analysis was based on delayed free recall performance: The number of freely recalled words (trial 7) as well as relative delayed recall performance as compared to the last learning trial (trial 5 minus 7). These parameters have shown to be sensitive to left temporal lobe dysfunction, especially left mesial-temporal pathology and left-sided temporal lobe surgery (45–47).

In order to assess the function of the non-dominant temporal lobe, a figural learning and memory test was used [DCS = Diagnosticum fuer Cerebralschaedigung revised, (48)]. The DCS tests learning and immediate recall of nine abstract designs consisting of five lines in five consecutive trials. Patients are asked to reproduce the designs from memory using five small sticks. Figural learning performance (sum of trials 1 to 5) and figural memory capacity (last learning trial) have been demonstrated to assess functions of the right temporal lobe, including hippocampal functions (49).

Verbal and non-verbal memory performance was assessed pre-surgically as well as approximately 1 year after surgery. Parallel test versions were used in the examination after surgery.

Z-scores were computed based on the VLMT- and DCS raw values and age-matched normative data retrieved from the test manuals. In order to examine post-surgical memory outcome, z-score differences between pre- and post-surgical memory performance were computed. A decrease in z-scores of more than one (standard deviation) was defined as deterioration.

Statistical Analyses

Three different ripple groups were defined, according to their association with IES and sleep spindles: (1). Overall ripples numbers (= All ripples), (2). ripples co-occurring with IES in the same iEEG channels (= IES ripples) and (3). spindle coupled ripples, i.e. ripples coincident with scalp spindles AND coupled to spindle troughs (=Spindle ripples). In all analyses, these three ripple types were observed separately.

In each patient, the correspondent memory-test results (z-score) were assigned to the respective mean mesial-temporal ripple rate, i.e. verbal memory performance to the left (para)hippocampal ripple rates and non-verbal memory performance to the right (para)hippocampal ripple rates (provided a left-sided speech dominance). Analyses were performed for verbal and non-verbal memory separately. Memory performance with a pre-surgical z-score $< -1{,}0$ was evaluated to be "impaired", and z-scores of -1 and larger as "healthy".

Three analyses were performed:

1. Mann–Whitney-U-Tests (two-sided) comparing ripple rates between patients with healthy and impaired memory.
2. Spearman correlations (one-sided) between the different ripple rates and the respective pre-surgical verbal or non-verbal memory performance.
3. Spearman correlation analysis of non-SOZ *vs.* SOZ ripples and verbal memory performance.
4. "Delta analysis" using Spearman correlations between the different ripple rates and the difference ("delta") of post- and pre-surgical memory results (post- minus pre-surgical z-scores).
   a) For all patients with left-sided temporal surgery.
   b) For patients in whom a surgical intervention had led to a removal of a left-sided ("verbal memory") hippocampus.

As the attribution of non-verbal memory is questioned, we concentrated on the well-established attribution of verbal memory performance to ripples in iEEG channels in the speech-dominant hippocampus in the "Delta analysis" (for further details, see Discussion).

The significance level of all analyses was $\alpha = 0.05$.

RESULTS

Patients and Clinical Data

A total of 25 patients could be included. Two patients had to be excluded because of a low EEG sample rate and four patients because of artifact-impaired scalp EEG, which prevented spindle analysis. **Table 1** offers an overview of the clinical data and implantation sites of the included patients. Two patients had seizures starting bilaterally in the mesial-temporal regions, 17 patients had seizures starting in one mesial-temporal region and six patients had seizure onset in the temporal lobe outside of mesial-temporal regions. Eleven patients received right-sided and six patients received left-sided amygdalohippocampectomy. Eight patients received temporal lobe resections without removing the mesial-temporal structures (four patients: left side, four patients: right side).

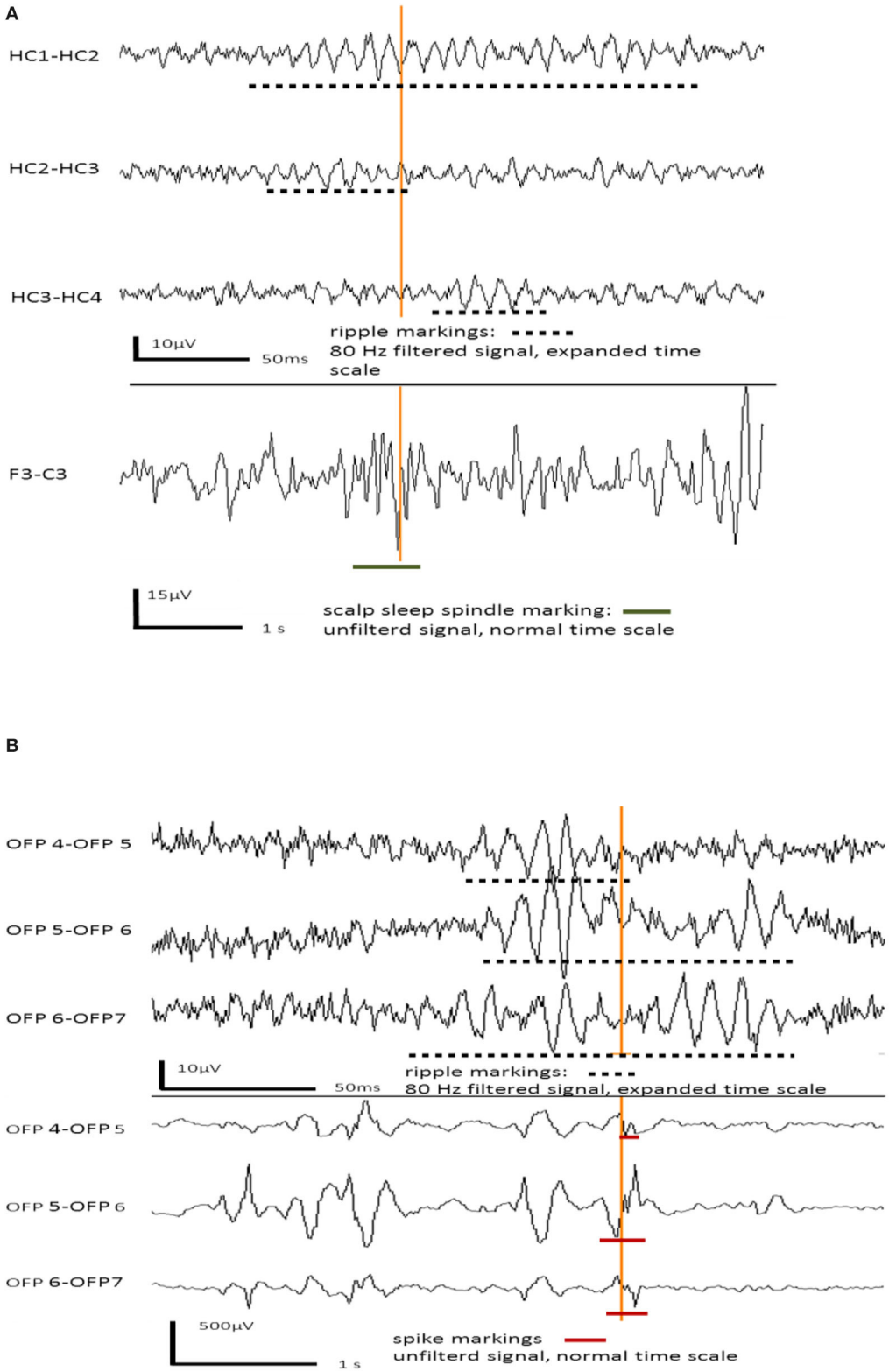

FIGURE 1 | (A) Example for hippocampal ripples in intracranial EEG channels co-occurring with a frontal sleep spindle in scalp EEG. **(B)** Example for hippocampal ripples co-occurring with interictal epileptic spikes in the same intracranial EEG channels.

TABLE 1 | Clinical data.

Patient	Age	Gender	Seizure type	MRI	Surgery	SOZ	Implantation
1	12	m	FAS, FIAS	tuberous sclerosis	R TL res & SAH	R mTL	R-HC, R-A, R-PHC, R-P, R-F
2	54	m	FIAS, FBTCS	L T-pole MEC, parenchymal lesions R F-bas & L-T-pole	L T-pole res	L mTL	L-HC, L-A, L-PHC
3	33	f	FAS, FIAS, FBTCS	no lesion	R tailored TL res	R TL w/o mTL	R-HC, R-A, R-PHC
4	47	m	FAS, FIAS	BL periventricular heterotopia, L HS	L SAH	L mTL	L-HC, L-A, L-PHC, L-O
5	49	m	FAS, FIAS	no lesion	R SAH	R mTL	L-HC, L-A, L-PHC, R-HC, R-A, R-PHC, R-F
6	53	m	FAS, FIAS, FBTCS	suspected FCD in R T-pole	R T-pole res & SAH	R mTL	R-HC, R-A, R-PHC, R-F
7	27	m	FAS, FBTCS	no lesion	R STG res	R STG	R-HC, R-A, R-PHC, R-F
8	33	m	FAS, FIAS, FBTCS	L mTL FCD	L post HC res & lesionectomy	L mTL	L-HC, L-A, L-PHC, L-F
9	52	f	FAS, FIAS, FBTCS	no lesion	L HC res & lesionectomy	L mTL	L-HC, L-A, L-PHC
10	55	f	FIAS, FBTCS	no lesion	L TL res & SAH	L mTL	L-HC, L-A, L-PHC, L-F, L-O
11	60	f	FAS, FIAS, FBTCS	BL T-pole MEC, L T-pole P lesion	L T-pole res	L T-pole	L-HC, L-A, L-PHC, R-PHC, R-A
12	28	f	FAS, FIAS	no lesion	L SAH	L mTL	L-HC, L-A, L-PHC
13	23	f	FAS, FIAS, FBTCS	R HS	R T-pole res & SAH	R & L mTL, T-pole	L-HC, L-A, L-PHC, R-HC, R-A, R-PHC
14	23	f	FAS, FIAS, FBTCS	no lesion	L SAH	L mTL	L-HC, L-A, L-PHC
15	37	f	FAS, FIAS, FBTCS	R T-pole MEC	R T-pole res	R T-pole	R-HC, R-A, R-PHC, R-T-pole
16	34	m	FAS, FIAS, FBTCS	L T-pole MEC	L T-pole res	L T-pole	L-HC, L-A, L-PHC
17	12	m	FAS, FIAS	suspected FCD in R T-pole	R TL res & SAH	R mTL	R-HC, R-A, R-PHC, R-F
18	21	f	FAS, FIAS, FBTCS	PCA WHO°I	R TL res & SAH	R mTL	R-HC, R-A, R-PHC, R-F
19	34	f	FAS, FIAS, FBTCS	ganglioglioma WHO°I	L FL/TL res & SAH	L FL, TL, mTL	L-HC, L-A, L-PHC, L-F, R-HC, R-A, R-PHC
20	48	m	FAS, FIAS, FBTCS	R F DVA	R O T res	R T O res	L-HC, L-A, L-PHC, R-HC, R-A, R-PHC, R-T, R-O
21	31	f	FAS, FIAS	BL HS	R TL res & SAH	R mTL	R-HC, R-A, R-PHC
22	40	f	FAS, FIAS, FBTCS	R HS	R TL res & SAH	R mTL	R-HC, R-A, R-PHC
23	44	f	FAS, FIAS, FBTCS	BL T P MEC	L T-pole res	L T-pole	L-HC, L-A, L-PHC, R-HC, R-A, R-PHC
24	45	m	FIAS, FBTCS	no temporal lesion	R TL res & SAH	R & L mTL	L-HC, L-A, L-PHC, R-HC, R-A, R-PHC
25	22	m	FAS	R HS	R SAH	R mTL	R-HC, R-A, R-PHC, R-F, R-P

Abbreviations: *A, Amygdala; AC, arachnoid cyst; SAH, selective amygdalohippocampectomy; bas, basal; BL, bilateral; DVA, developmental venous anomaly; FL, frontal lobe; FAS, focal aware seizure; FBTCS, focal to bilateral tonic-clonic seizure; FCD, focal cortical dysplasia; FIAS, focal impaired awareness seizure; HC, hippocampal, HS, hippocampal sclerosis; L, left; MEC, meningoencephalocele; mTL, mesial temporal lobe; O, occipital; P, parietal; PCA, pylocytic astrocytoma; PHC, parahippocampal; post, posterior; R, right; res, resection; STG, superior temporal gyrus; T , temporal; TL, temporal lobe.*

Memory Performance

In pre-surgical memory testing nine patients had significant non-verbal memory impairment (Z-score $< -1{,}0$) with intact verbal memory, one patient had verbal memory impairment with intact non-verbal memory and four patients had both verbal and non-verbal memory impairment.

Of the 11 patients with right-sided amygdalohippocampectomy, seven patients had similar memory performance outcomes and one patient had a post-surgical deterioration of non-verbal memory function. Two patients benefitted from surgery with amelioration of non-verbal memory function.

Of the six patients with left-sided amygdalohippocampectomy, one patient had a similar memory performance outcome and three patients had deterioration of verbal memory function (two patients in verbal and one patient in verbal and non-verbal memory). In two patients with left-sided amygdalohippocampectomy a significant amelioration of non-verbal memory was observed, whilst verbal memory remained impaired in these patients. **Table 2** gives an overview of the memory performance results of the cohort.

Descriptive Ripple Statistics

Ripples, IES-associated ripples, and spindle-coupled ripples were found in all patients. In total, 231 mesial-temporal channels were analyzed, including 95 SOZ channels and 136 channels outside the SOZ (NSOZ). The following ripple rates are given as mean $\pm$ standard deviation with the interquartile ranges in brackets. Considering all channels, rates of 14.1 $\pm$ 9.5/min (15,0/min) overall ripples, 6.2 $\pm$ 6.1/min (9.0/min) IES ripples and 0.9 $\pm$

TABLE 2 | Memory performance results.

Patient	Speech	IQ	Pre VM	Pre NVM	Post VM	Post NVM	Surgery
1	L	N.A.	healthy	impaired	healthy	impaired	R TL incl MTL
2	L	109	healthy	impaired	healthy	healthy	L T-pole (MTL pres)
3	L	104	healthy	healthy	healthy	healthy	tailored TL res
4	L	100	impaired	impaired	impaired	healthy	L sAHE
5	L	107	healthy	healthy	healthy	healthy	R sAHE
6	L	N.A.	healthy	healthy	healthy	impaired	R T-pole res & AHE
7	L	124	healthy	healthy	healthy	healthy	R STG res
8	L	94	healthy	healthy	impaired	healthy	L post HC res & lesionectomy
9	L	118	healthy	impaired	impaired	impaired	L HC res & lesionectomy
10	L	111	impaired	impaired	impaired	impaired	L TL res & sAHE
11	L	100	healthy	impaired	impaired	N.A.	L T-pole res
12	L	101	healthy	healthy	impaired	impaired	L sAHE
13	L	93	healthy	impaired	healthy	impaired	R T-pole res & AHE
14	L	92	impaired	impaired	impaired	healthy	L sAHE
15	L	N.A.	healthy	healthy	healthy	healthy	R T-pole res
16	L	95	healthy	N.A.	healthy	healthy	L T-pole res
17	L	N.A.	healthy	healthy	healthy	healthy	R TL res & AHE
18	L	121	healthy	healthy	healthy	healthy	R TL res & AHE
19	L	81	impaired	impaired	impaired	impaired	R TL res & AHE
20	L	143	healthy	healthy	healthy	healthy	R O T res
21	L	104	healthy	impaired	healthy	impaired	R TL res & AHE
22	L	N.A.	healthy	impaired	healthy	healthy	R TL res & AHE
23	L	93	healthy	impaired	healthy	impaired	L T-pole res
24	L	N.A.	healthy	impaired	healthy	N.A.	R TL res & AHE
25	L	97	impaired	healthy	healthy	healthy	R sAHE

Abbreviations: *L, left; rem mTL, remaining mesiotemporal structures; (m)TL, (mesial) temporal lobe; N.A., not available; NVM, Nonverbal Memory; Pre, pre-surgical; Post,post-surgical; VM, Verbal Memory; R,right.*

1.0/min (0.7/min) spindle coupled ripples and 7.9 ± 5.0/min (7.1/min) isolated ripples (without any co-occurrence with sleep-spindles or IES showed) were detected.

In SOZ channels rates of 18.5 ± 1.5/min (9.1/min) overall ripples, 9.1 ± 6.8/min (10.8/min) IES ripples and 1.2 ± 0.7/min (1.2/min) spindle coupled ripples were detected. In NSOZ channels rates of 11.0 ± 8.0/min (11.9/min) overall ripples, 4.3 ± 5.0/min (6.1/min) IES ripples and 0.8 ± 0.7/min (0.7/min) spindle coupled ripples were detected. The rates of all three ripple types were significantly higher in SOZ channels than in NSOZ channels ($p < 0.001$ for each ripple type).

Comparison Pre-surgical Memory Impairment and Ripple Rates

To examine a connection between pre-surgical memory impairment and mesial-temporal ripple rates, patients were divided into two groups (healthy vs. impaired). Impaired memory was defined by a memory performance z-score under −1,0. Mann–Whitney-U-Tests were computed separately for verbal and non-verbal memory performances, attributing left mesial-temporal ripple rates to verbal memory and right mesial-temporal ripple rates to non-verbal memory.

Regarding verbal memory and left-sided mesial-temporal ripple rates, significantly higher overall ripple rates were seen in patients with impaired verbal memory than in patients with healthy memory ($p = 0.039$). Regarding IES ripples, higher rates were also seen in patients with impaired verbal memory, but the difference was not significant ($p = 0.078$). Regarding spindle ripples, no differences between the two groups were seen (see **Figure 3A**).

Regarding non-verbal memory and right-sided mesial-temporal ripple rates, no differences between the two groups were seen in all three ripple types (All Ripples: $p = 0.606$; IES Ripples: $p = 0.541$; Spindle Ripples: $p = 0.423$) (see **Figure 3B**).

Correlation Analysis of Pre-surgical Memory Performance and Ripple Rates

This analysis was performed to investigate the assumed correlation of ripples, especially spindle-ripples, and pre-surgical memory performance.

Spearman correlations of left mesial-temporal ripple rates and pre-surgical verbal memory results showed a highly significant negative correlation of the overall ripple rate and pre-surgical verbal memory performance (z-scores) was seen ($r_s = -0.475$; $p = 0.006$) (see **Figure 4A**). For IES ripples, a negative correlation with pre-surgical memory was seen as well ($r_s = -0.458$; $p = 0.007$) (see **Figure 4B**), whereas there was no significant correlation between spindle ripples

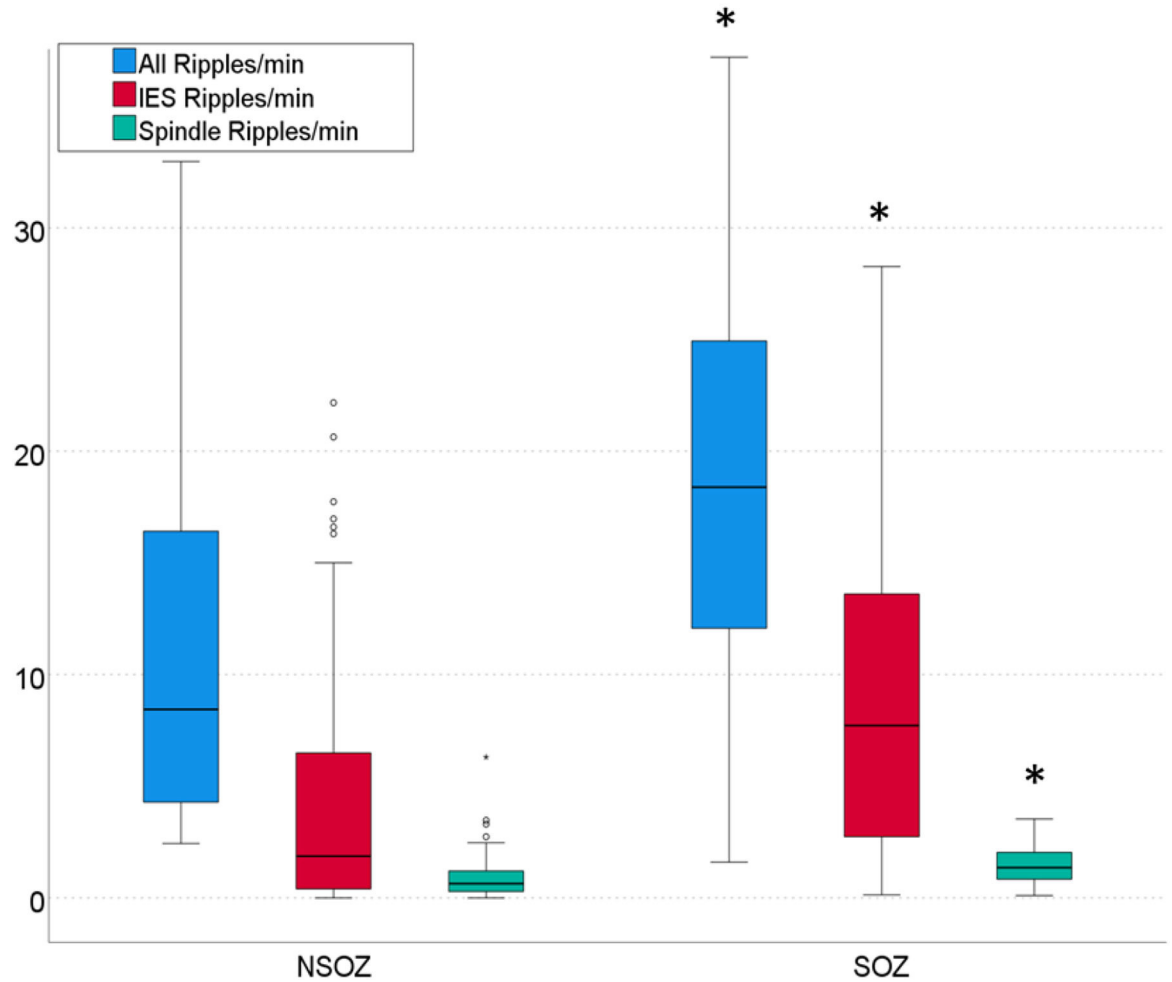

FIGURE 2 | Rates of three ripple types per min in channels outside the seizure onset zone (NSOZ) compared to ripples in channels inside the seizure onset zone (SOZ). Significantly higher overall ripple, IES ripple, and Spindle ripple rates were found in SOZ channels ($^{*}p < 0.001$, respectively).

and verbal memory performance ($r_s = -0.194$; $p = 0.149$) (see **Figure 4C**).

Spearman correlations of right mesial-temporal ripple rates and pre-surgical non-verbal memory results (Z-scores) showed no correlation regarding overall ripples ($r_s = 0.160$; $p = 0.540$) and IES ripples ($r_s = 0.119$; $p = 0.377$). No significant correlation for spindle ripples was seen either ($r_s = 0.324$; $p = 0.204$).

Correlation Analysis of Non-SOZ vs. SOZ Ripples and Verbal Memory Performance

Focussing on the verbal side hippocampal SOZ channels, Spearman correlations were performed regarding a possible connection of different ripple events and z-values of verbal memory performance. In this analysis, no significant correlation was seen for spindle ripples as expected ($rs = -0.144$, $p = 0.337$. Regarding overall ripples and IES ripples medium strength negative correlations of ripple rates and memory performance were seen that were not (but tendentially) significant (overall ripples: $rs = -0.366$; $p = 0.086$; IES ripples: $rs = -0.370$; $p = 0.080$) (see **Figure 2**).

Delta Analysis (Correlation Analysis of Post- to Pre-surgical Verbal Memory Difference and Ripple Rates)

In the following analysis, we aimed to examine the influence of surgical removal of ripple generating tissue on post-surgical verbal memory performance. Therefore, Spearman correlations between the difference in post- to pre-surgical verbal memory performance and left-sided ripple rates were computed. The 16 patients with left-sided temporal or mesial-temporal surgery were included in this analysis. No significant correlation was seen regarding all three ripple rates (all ripples: $r_s = 0.092$; $p = 0.310$; IES ripples: $r_s = 0.059$; $p = 0.376$; spindle ripples: $r_s = 0.042$; $p = 0.411$).

In a sub-analysis only the six patients with left-sided amygdalohippocampectomy were considered. Again, no correlation between the difference of post- and pre-surgical

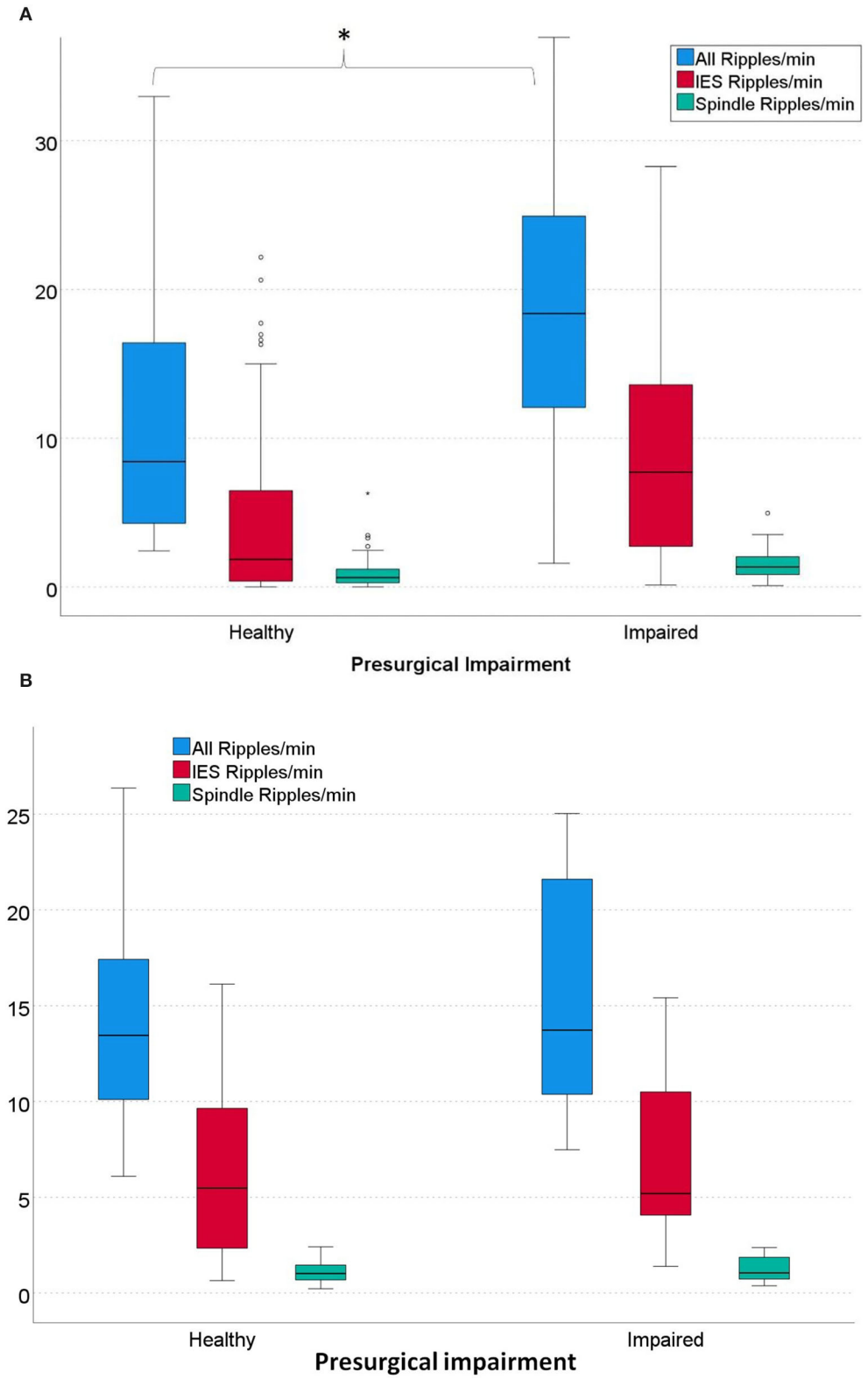

FIGURE 3 | (A) Rates of left-sided mesial-temporal ripples in patients with healthy *vs.* impaired pre-surgical verbal memory. Patients with impaired verbal memory had significantly higher overall ripple rates than patients with healthy memory. (All Ripples: $^*p = 0.039$; IES Ripples: $p = 0.078$; Spindle ripples: $p = 0.379$). $\alpha = 0.05$. **(B)** Rates of all right-sided mesial-temporal overall Ripples and IES Ripples in patients with healthy *vs.* impaired pre-surgical non-verbal memory. No significant differences were seen for all three ripple types (All Ripples: $p = 0.606$; IES Ripples: $p = 0.541$; Spindle Ripples: $p = 0.423$); $\alpha = 0.05$.

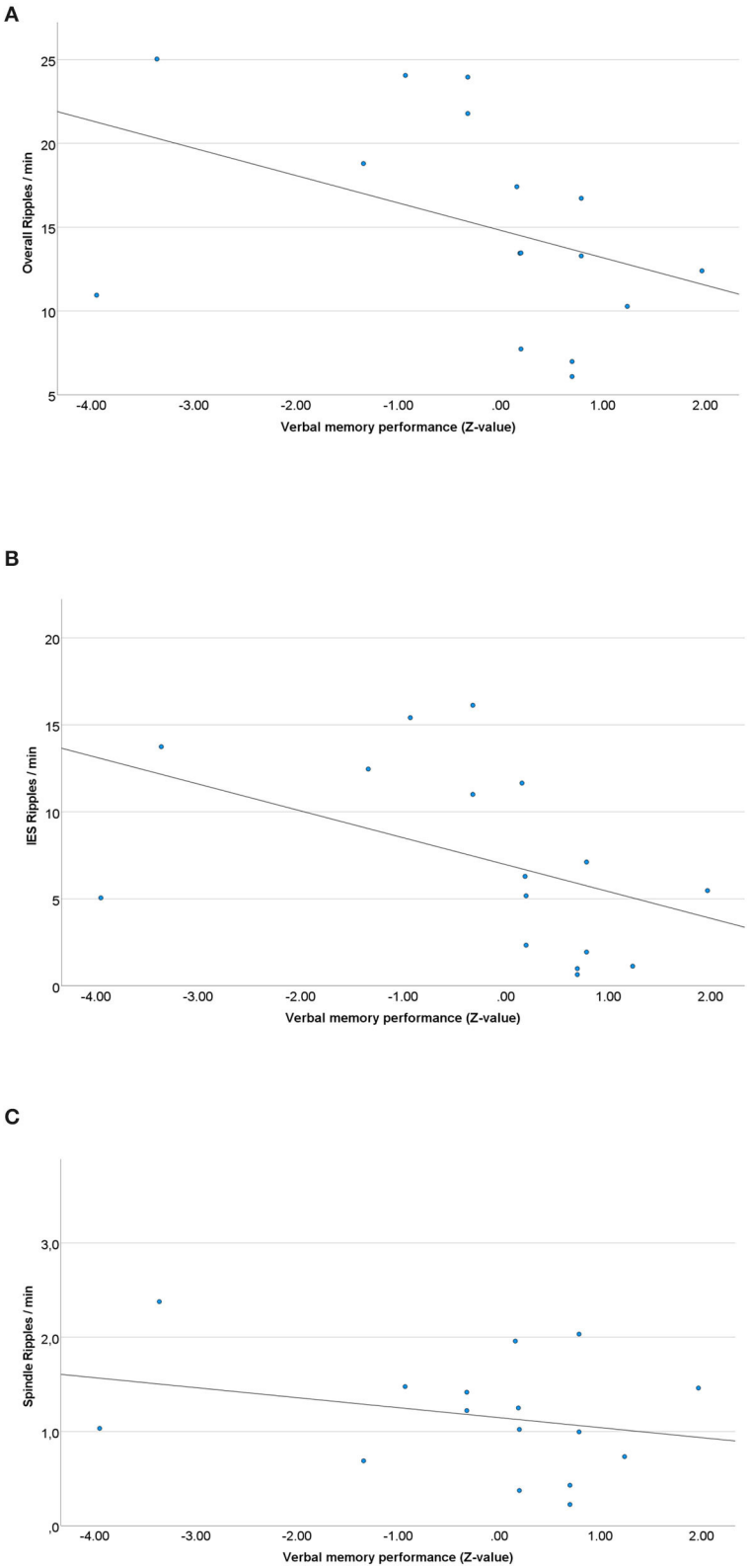

FIGURE 4 | (A) Scatterplot of the overall left mesial-temporal ripple rate and pre-surgical verbal memory results. A significant negative correlation between ripple rates and memory performance was seen ($r_s = -0.475$; $p = 0.006$). **(B)** Scatterplot of the left mesial-temporal IES ripple rate and pre-surgical verbal memory results. A significant negative Spearman correlation was seen ($r_s = -0.458$; $p = 0.007$). **(C)** Scatterplot of the left sided spindle ripple rate and pre-surgical verbal memory results. No significant Spearman correlation was seen ($r_s = -0.194$; $p = 0.149$).

memory performance [Δ = post-VLMT - pre-VLMT(Z-scores)] and the rates of all three ripple types was seen (all ripples: r_s = 0.127; p = 0.293; IES ripples: r_s = 0.018; p = 0.469; spindle ripples: r_s = 0.220; p = 0.174).

DISCUSSION

The main hypothesis of this study was that higher rates of mesial-temporal spindle-ripples correlate with good memory performance in epilepsy patients. In addition, we hypothesized that surgical removal of spindle-ripple-generating brain tissue would correlate with a decline in memory performance. In contrast, higher rates of ripples associated with IES were hypothesized to correlate with poor memory performance and the surgical removal of IES ripples would lead to an enhancement of memory performance.

A significant correlation between spindle ripples and pre-surgical verbal or non-verbal memory performance could not be found in this study. Removing tissue that generated high rates of spindle-ripples showed no correlation to decline of memory function. As hypothesized, high ripple rates of overall ripples and IES ripples were associated with poor memory performance. This indicates that the majority of ripples identified in our patients are linked to epileptic activity. Most likely the percentage of physiological spindle-ripples that contribute to memory function is too small compared to the overall ripple population and therefore hard to identify in mesial-temporal structures of patients with severe epilepsy.

Methodological Considerations

Ripple, IES, and sleep spindle detection were performed automatically by a published detector of our group (40). In addition, the first 5 min of each EEG segment were visually checked for artifacts. In contrast to time-consuming visual detection, this semi-automatic approach would be applicable for potential clinical use. We chose relatively short segments of N2 periods of slow-wave sleep, as an extension of record time would not lead to a higher share of spindle ripple rates and would have extended the difficulty of finding interictal periods outside of seizures and subclinical seizures.

In other studies observing physiological HFO activities, simultaneously performed neuropsychological tasks were used to induce or increase physiological HFO activity (22, 24, 50, 51). In this study, data was collected retrospectively and no specific memory tasks were conducted prior to the iEEG recording nights due to two reasons: On the one hand, it can be assumed that patients learn new memory content every day and spindle-ripple activity associated with memory consolidation can be observed without extra memory tasks. On the other hand, this study aimed to estimate the impact of physiological oscillations on the evaluation of a standard iEEG during the recording period, as this is the information needed by clinicians when evaluating ripples as markers of epileptogenic brain regions.

One challenge of the present study is to clearly separate areas that have a high function from those that generate seizures. Healthy and epileptic regions in patients with refractory epilepsy often overlap and seizures arise from hippocampi that can still have good remaining memory function. Usually, only patients with contradictory results in prior diagnostics (scalp EEG, MRI, neuropsychological tests) receive iEEG diagnostics. This leads to a pre-selection of patients with highly complicated epilepsies and often bilateral mesial-temporal involvement.

Especially in temporal lobe epilepsy, clinicians and researchers face an extensively complicated network disease (52–54). Bilateral independent seizures are frequent (55). Regularly, patients with unilateral surgical removal of the diagnosed epileptogenic zone do not experience seizure reduction after unilateral mesial-temporal lobe (MTL) resection (55–57). This suggests that epileptic regions could remain off the grid, e.g. one potential epileptic hippocampus could not be considered as part of the SOZ in the short period of iEEG registration. Furthermore, there are no secure healthy control regions, concepts of SOZ, and areas outside the SOZ used in this study are susceptible to errors and uncertainties. In our study, this is directly reflected in the results as it is challenging to separate ripples reflecting epileptic activity from those representing memory.

Regarding our delta analysis (analyzing pre- and post-surgical memory), we intended to obtain a preferably clear attribution of ripple generating tissue to a certain brain region. It has been discussed if the type of material used during a memory task as well as the underlying processing influence the functional asymmetry in the mesial-temporal lobe and therefore challenges the left-verbal/right-non-verbal dichotomy (58). Especially the attribution of the non-speech-dominant hippocampus to non-verbal memory is questioned (59). Therefore, we concentrated on the well-established attribution of verbal memory performance to ripples in iEEG channels in the speech-dominant hippocampus. In the cohort of this study all patients had left-sided language dominance, therefore only verbal memory test results and left hippocampal and parahippocampal channels were considered for the delta analysis.

We focused on ripple range HFO for this study, as we were specifically aiming at the co-occurrence between ripples and sleep spindles. No association of fast ripples with sleep-spindles, slow-waves, or another hint of physiological function in the mesial temporal regions has been reported in the literature so far. Most physiological fast ripples are described in paracentral and occipital regions. Also, this phenomenon has nearly exclusively been observed in the context of prior tasks or induction (22, 24).

This study concentrated on memory parameters that are associated with hippocampal memory functions. However, long-term memory consolidation is not only based on mesial temporal lobe function but also on an extensively and bilaterally distributed process (60). Long-term memory storage depends on a functioning precedent of short-term memory storage, e.g. the short-term maintenance of verbal memory occurs in left neocortical networks (left pre-central, supra-marginal, and inferior frontal gyri) (61). An impaired short-term memory process is a possible cause for impairment of long-term memory consolidation. However, the chronological sequence of the overall memory storage process was not investigated. As clinically guided electrode implantation leads to restricted registration areas, we concentrated on spontaneous ripple activity in mesial temporal areas and its attributed memory functions. Impaired

short-term memory therefore could be one reason, why a correlation between memory performance and spindle ripple rates was not found in this study.

In addition, the (long-term) influence of anti-epileptic drugs on memory capability is unclear. Most of the patients in iEEG studies have taken multiple anti-epileptic-drugs medications. This could be another circumstance that had a perturbing influence on this study.

Mesial-Temporal Physiological and Epileptic Ripples

Few studies have examined the interaction of memory performance and mesial-temporal ripples in general or spindle-associated ripples in particular. Axmacher and co-workers found an increase in overall ripple band HFO during cognitive tasks and were the first to find a correlation between ripple rates and memory performance in humans (50). A recent study found a correlation between the amount of spindle coincident ripples after a spatial navigation task in two subsequent iEEG recording nights (51). Thus, in addition to the knowledge of spindle-associated ripple activity (33, 35, 62), there seems to be strong evidence that physiological ripples can be observed in mesial-temporal structures of patients with epilepsy. However, a correlation between spindle-trough-coupled ripples and memory function could not be seen in the current study. Nevertheless, it seems likely, that the detected spindle ripples mirror physiological HFO activity, as the majority of spindle coincident ripples have shown to have different amplitude features and are coupled to spindle troughs (33, 42).

However, it has to be considered that sleep spindles and sharp wave ripples might be pathologically compromised in patients with epilepsy. Transformation of physiologically sharp-wave-ripple complexes or possible pathological hippocampal sleep-spindle activity leading to interictal epileptiform discharges (IED) has been reported in some patients (63, 64). Frauscher and co-workers have particularly observed the relationship between mesial-temporal epilepsy and sleep-spindle expression, showing that hippocampal spiking correlates with lower rates of hippocampal sleep-spindles. The authors suggested that epileptic discharges might be transformations of physiological spindle activity in patients with mesial-temporal lobe epilepsy (65). In the current study, one patient was excluded because of pathologically changed sleep-spindles with sharp transients. In all other patients, sleep-spindles had typical shapes. When pathologically "distorted" spindles are observed, spindle-ripple analysis seems not to be reasonable. However, epileptically compromised sleep-spindle activity seems to be rare. The findings of Frauscher and co-workers might suggest, that in patients with epilepsy the proportion of spindle-ripples might not only be much lower compared to epileptic ripple activity but might also be suppressed by it.

We found a clear negative correlation between pre-surgical memory performance and overall mesial-temporal ripple rates (i.e. irrespective of any coincidence with spindles or IES). A former study showed similar results: Jacobs and co-workers found no correlation between overall HFO or ripple activity and memory performance. On the contrary, a high number of HFO or ripples correlated with poor memory performance outside the SOZ, whereas patients with a good memory performance showed less frequent HFO in channels outside the SOZ (32). It seems that the majority of mesial-temporal ripples in epilepsy patients reflect the pathological activity. In the current study, the share of spindle-ripples was marginal compared to overall ripple rates and much lower than IES-associated ripples. As we could not find any correlation between spindle-ripples and memory, it seems likely, that epileptic ripple activity impairs physiological activity immensely as they co-occur close-by both in time and place, separating these activities.

Removing tissue that showed high rates of spindle ripples prior to resection showed no correlation to a decline of post-surgical memory function in our two groups of patients with left-sided temporal surgery or mesial-temporal surgery. This goes in hand with the findings of a recent study which showed that the value of spindle-ripples alone to identify physiological ripples on pre-surgical diagnostics is limited (66).

CONCLUSION

This study fails to establish a clear link between memory performance and spindle-ripples. This highly suggests that spindle-ripples are only a small portion of physiological ripples contributing to memory performance. Standardized neuropsychological tests likely measure functional results of all ripples and might not be sensitive enough to detect changes in spindle-ripple occurrence alone. Moreover, high numbers of epileptic ripples might prevent us from seeing the effect of ripple numbers on memory, especially in patients with bilateral disease and very active epilepsy. However, an important take home message is that our results suggest that spindle ripples do not necessarily compromise the predictive value of ripples in patients with temporal epilepsy. To come closer to our aim of measuring memory function and epileptic activity with HFO, measures beyond spindle-ripples should be evaluated. A combined analysis that includes all known characteristics of physiological ripples, their coupling to sleep spindles and slow waves (67, 68), and additionally considering hippocampal theta waves and other iEEG features (69, 70) might show a better correlation between these ripples and memory function.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Committee of the Freiburg University Medical Center. Written informed consent to participate in

this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

JJ, KW, and JB have contributed to the conception and design of the study. JB, KW, and DL-P have contributed to the acquisition and analysis of data. JB, KW, KK, and JJ have drafted significant portions of the manuscript and figures. AS-B provided all clinical data and EEG data. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2022.876024/full#supplementary-material

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Verbal Learning and Longitudinal Hippocampal Network Connectivity in Temporal Lobe Epilepsy Surgery

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Introduction: Learning new verbal information can be impaired in 20–40% of patients after mesial temporal lobe resection. In recent years, understanding epilepsy as a brain network disease, and investigating the relationship between large-scale resting networks and cognition has led to several advances. Aligned studies suggest that it is the integrity of the hippocampal connectivity with these large-scale networks what is relevant for cognition, with evidence showing a functional and structural heterogeneity along the long axis hippocampus bilaterally.

Objective: Our aim is to examine whether pre-operative resting-state connectivity along the long hippocampal axis is associated with verbal learning decline after anterior temporal lobe resection.

Methods: Thirty-one patients with epilepsy who underwent an anterior temporal lobe resection were pre-surgically scanned at 3-tesla, and pre/post-surgery evaluated for learning deficits using the Rey Auditory Verbal Learning Task (RAVLT). Eighteen controls matched by age, gender and handedness were also scanned and evaluated with the RAVLT. We studied the functional connectivity along the (anterior/posterior) long axis hippocampal subregions and resting-state functionally-defined brain networks involved in learning [executive (EXE), dorsal attention (DAN) and default-mode (DMN) networks]. Functional connectivity differences between the two groups of patients (learning intact or with learning decline) and controls were investigated with MANOVA and discriminant analysis.

Results: There were significant differences in the pattern of hippocampal connectivity among the groups. Regarding the anterior connectivity hippocampal pattern, our data showed an increase of connectivity in the pathological side with the DAN ($p = 0.011$) and the EXE ($p = 0.008$) when comparing learning-decline vs. learning-intact patients. Moreover, the non-pathological side showed an increase in the anterior connectivity pattern with the DAN ($p = 0.027$) between learning-decline vs. learning-intact patients. In contrast, the posterior hippocampus showed a reduction of connectivity in the learning-decline patients with the DMN, both in the pathological ($p = 0.004$) and the non-pathological sides ($p = 0.036$). Finally, the discriminant analysis based on the

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pre-operative connectivity pattern significantly differentiated the learning-decline patients from the other groups ($p = 0.019$).

Conclusion: Our findings reveal bilateral connectivity disruptions along the longitudinal axis of the hippocampi with resting-state networks, which could be key to identify those patients at risk of verbal learning decline after epilepsy surgery.

Keywords: resting-sate fMRI, verbal learning, temporal lobe epilepsy, DMN (default mode network), dorsal attention network (DAN)

INTRODUCTION

Temporal lobe surgery is an effective treatment in drug-resistant seizures in temporal lobe epilepsy (TLE) (1). However, patients are at risk of verbal learning impairment; assessed through the Rey Auditory Verbal Learning Task (RAVLT), up to 20–40% of patients can be impaired, as the resection affects mostly the anterior part of the mesial temporal lobe, including the hippocampus, which is fundamental in memory and learning (2).

The hemispherical side of the surgery, the resection extent, the verbal learning skills prior to surgery, or the age of seizure's onset are factors modulating the risk of suffering this decline (3). However, not all patients with epilepsy show verbal learning deficits postoperatively, in that some patients perform similarly before and after surgery (4). Such findings indicate that some patients have the neuroplastic potential to compensate for the resected area, leading to post-operative preservation.

Different neural mechanisms might explain the individual differences in the maintenance of verbal learning despite seizure-related and resection-related damage. Evidence suggests that these mechanisms may lead to changes in the connectivity pattern of different neural networks to cope with the impact of pathology and epileptic seizures. In this sense, disruption of neural networks in TLE patients have been reported to be related with cognitive functions (5). More specifically, a decrease in functional connectivity between the hippocampus and the posterior cingulate cortex (PCC), a key hub in the Default Mode Network (DMN) has been consistently reported in relation to verbal memory deficits (5, 6).

Both up-regulation and down-regulation of functional connectivity can be found in patients with TLE (7). Using graph theory measures, a decrease in nodal efficiency of the left hippocampus was related to impairment of verbal memory in patients with left TLE, whereas increases in nodal efficiency of the inferior frontal gyrus and the supplementary motor area were correlated to semantic and phonological fluency in patients with right TLE (8). For those undergoing surgery, down-regulation ipsilateral to the affected side coupled with concurrent contralateral up-regulation of functional connectivity has been associated with a preserved cognitive outcome after the procedure (9). In this vein, higher connectivity of the pathological hippocampus with the PCC was associated with a high risk of post-surgical decline, whereas the connectivity of the contralateral hippocampus to the PCC related to less risk of decline (6). Finally, another study using graph-theory measures (efficiency, integration, and centrality), reported an enhanced integration of the contralateral hippocampus to be predictive of cognitive preservation after surgery, and increased connectivity in the inferior frontal gyrus was related with preserved performance of language tasks (10). In summary, evidence suggest a bilateral reorganization of network connectivity involving both hippocampi and extra hippocampal structures in patients with TLE, that is related to verbal memory and post-surgical decline.

Further evidence also showed relevant changes along the longitudinal axis in the to-be resected hippocampus. A shift in hippocampal activation during an encoding task from the anterior regions toward more post-surgically preserved posterior regions of the to-be resected hippocampus conferred protection against verbal memory impairment after surgery (11). This would suggest some level of reorganization also along the long hippocampal axis associated with decline after resection, yet their underlying neural mechanisms remain poorly understood. In terms of the functional heterogeneity of the anterior and posterior hippocampus and their involvement in cognitive functions, aligned animal and human neuroimaging studies suggest that cognitive domains superimpose according to a functional gradient along the longitudinal axis (12), which also exhibits differences in functional connectivity in TLE patients (13).

Approaching TLE as a brain network disease (14) has been a framework hypothesis that has improved the understanding of seizures, EEG and MRI findings in patients with epilepsy (15). Noteworthy, evidence from recent years indicate that it is the integration of the hippocampi with bilateral, large scale networks what is relevant for cognitive function (16, 17); these large-scale networks can be measured reliably at rest (18), providing a useful tool for connectivity analysis. Network disruption has proven to be the key underlying some neurological deficits (19), and might be a suitable candidate to predict neuropsychological impairment that is less dependent on lesions in TLE (20). Moreover, differences in connectivity and functionality along the longitudinal axis of the hippocampal formation have revealed two systems (21). Despite both systems playing a role in learning and retrieval tasks, it has been hypothesized that they differ in the connectivity with different large-scale networks, with the anterior portion closely connected to the Dorsal Attention (DAN) and Executive Networks (EXE) and related to encoding of external stimuli, while the posterior portion is more closely connected with the DMN and related to memory retrieval and internal sources of information (22). These large-scale networks involve

mainly fronto-parietal areas, and their relation to cognitive functions has long been stablished (23, 24).

In the current study, we have investigated the impact of the bilateral functional connectivity along the longitudinal axis of the hippocampus with these large-scale networks and how this could relate to the risk of verbal learning impairment seen in post-surgical epilepsy patients. Of note, the above-mentioned studies in TLE patients evaluated the connectivity of the hippocampus as a whole. However, the longitudinal axis of the hippocampus displays differences in connectivity with large scale networks, and this fact relates to the cognitive functions supported (13, 21, 22, 25). More specifically, we focused on verbal learning abilities that could be impaired after surgery assessed through RAVLT, since previous studies have demonstrated deficits in patients undergoing temporal lobe surgery (2). Verbal learning requires different cognitive processes to be intact in order to be performed correctly. The RAVLT evaluates verbal learning requiring both retrieval (26) and encoding processes, as well as attentional shifting strategies (27) and executive functioning (28). These processes that are involved in verbal learning require the activation of both the anterior and posterior hippocampal formation.

Specifically, we compared the pre-surgical connectivity of both hippocampi between patients who had an impairment in the RAVLT learning domain after surgery with those who had not. We hypothesized that functional connectivity of the anterior and posterior hippocampal formation with DMN, EXE, and DAN differ in patients with verbal learning impairment after mesial temporal resections already before epilepsy surgery. We also analyzed this connectivity for the hippocampus as a whole, to investigate whether the anterior/posterior division provided further information on these patients. This study offers the possibility to identify biomarkers that predict the prognosis of the surgical outcome, which may be key for the pre-surgical planning.

METHODS

Participants

We included 31 consecutive patients who underwent an anterior temporal lobe resection for epilepsy surgery in the period 2009–2014. All patients were operated by the same neurosurgical team, and all the tissue was analyzed in the neuropathology department of our hospital. We also scanned 18 controls matched by age (T-student = 0.15, $p = 0.516$), gender ($X^2 = 0.44$, $p = 0.834$) and handedness ($X^2 = 0.016$, $p = 0.9$).

From the 31 patients, 18 were women (58.1%), with a median age of 49 years old (range 44 years). Sixteen patients (51.6%) had a left mesial temporal lobe resection, and 24 (77.4%) had signs of hippocampal sclerosis on the tissue sample. After surgery, 14 patients (45.2%) remained completely seizure free during follow-up (median time of follow-up 76 months, range 55 months). There was no difference among patient's groups in terms of seizure freedom after surgery (learning decline 45.5% of seizure freedom vs. learning intact 60% of seizure freedom, $X^2 = 0.61$, $p = 0.436$). See **Table 1** for demographic details of the patients and healthy control group samples. The study was conducted in accordance with the principles of the Declaration of Helsinki. Written informed consent was obtained for every participant, and the study was approved by the Clinical Research Ethics Committee of Bellvitge University Hospital.

Verbal Learning Assessment

All patients were assessed using the RAVLT (29) before and after surgery for evaluation of cognitive functioning. The stimuli that were presented in each of the evaluation were different. The test was performed in a median time of 9 months (range 5–19 months) before surgery, while the evaluation after surgery was performed in a median time of 6 months (range 4–12 months). Between both tests the span of time was a median of 17 months (range 11–28 months). In this task, the evaluator reads 15 different words to the patients. In an immediate recall test, patients are asked to repeat after each list all the words they could remember, regardless of the order in which they were presented by the experimenter. This task was repeated five times in a row. The final performance in the five lists was recollected as the absolute number of words remembered in the last run for each subject. This final score as an absolute number of words was used as a measure of verbal learning performance. Then, this score was transformed into standardized values using normative data from Hispanic cohorts, corrected for age, gender and educational level (30).

MRI Data Acquisition

Patients underwent a pre-operative whole-brain structural MRI scans using a 3.0 Tesla Siemens Trio MRI. A 32-channel phased-array head coil system was used to acquire high-resolution T1-weighted images (slice thickness = 1 mm; no gap; number of slices = 240; TR = 2,300 ms, TE = 3 ms, matrix = 256 × 256; FOV = 244 mm; voxel size 1 × 1 × 1 mm). Resting state fMRI data were collected using a single-shot T2*-weighted gradient-echo EPI sequence (slice thickness = 4 mm; no gap; number of slices = 32, interleaved order; TR = 2,000 ms; TE = 29 ms; flipangle = 80°; matrix = 80 × 80; voxel size = 3 × 3 × 4 mm^3, 110 volumes). During the resting state, participants were instructed to keep still with the eyes closed but not fall asleep, and to not focus on any thoughts, as far as possible. Healthy participants underwent the same neuroimaging protocol.

Hippocampal Volumes

Total hippocampal volumes were segmented from a fully automated pipeline for hippocampal subfields including the automated cortical parcellation and subcortical recon-all tools implemented in FreeSurfer 6.0 (http://surfer.nmr.mgh.harvard.edu/). The technical details have been described previously (31). To adjust for differences in head size, all volumes were normalized to total intracranial volume (ICV) by dividing by intracranial ICV calculated using FreeSurfer. Finally, all generated hippocampal images were visually inspected to ensure there were no technical failures or mislabeling.

Resting-State Functional Connectivity Analysis

Independent Component Analysis (ICA) was used to delineate spatially independent and temporally coherent patterns of

TABLE 1 | Sociodemographic and clinical characteristics of Temporal Lobe Epilepsy patients and controls.

	Learning decline $N = 11$	Learning intact $N = 20$	Controls $N = 18$	*p*
Age (years)	47 (7.5)	51.7 (12.1)	49.5 (11.9)	0.516
Sex (female)	6 (54.5%)	12 (60%)	11 (61.1%)	0.937
Age at onset (years)	18.1 (13.3)	12.6 (10.4)		0.207
Left resection (participants)	8 (72.7%)	8 (40%)		0.081
HS (participants)	8 (72.7%)	16 (80%)		0.643
Seizure-free* (participants)	6 (54.5%)	8 (40%)		0.477
Post-surgical follow-up**	86 (50)	93.5 (55)		0.919
Hipp volume (cm^3)				
Pathological	2.77 (0.5)	2.8 (0.5)	3.33 (0.3)	**<0.001**
Contralateral (Participants)	3.41 (0.2)	3.52 (0.2)	3.41 (0.3)	0.154
RAVLT pre	12.2 (2.2)	11.1 (2.2)	11.7 (2.4)	0.472
RAVLT post	8.2 (1.2)	11.5 (2.7)	11.7 (2.8)	**0.002**

Data presented as number (percentage). N, number of participants; HS, Hippocampal Sclerosis; Hipp, Hippocampus; RAVLT, Rey Auditory Verbal learning Test.
**At the end of follow-up.*
***In months, median (range). Significance assessed with the Mann-Whitney test. The bold values indicate the statistically significant.*

functional brain connectivity in the resting state DMN, EXE and DAN.

Individual functional data pre-processing was carried out using Statistical Parameter Mapping software (SPM12, Welcome Department of Imaging Neuroscience, University College, London, UK, http://www.fil.ion.ucl.ac.uk/spm/) following the standard protocol. The preprocessing of the data included realignment, co-registration between the structural T1 and their respective mean functional image, normalization, spatial smoothing (FWHM 8 mm). Then, to extract the different functional networks by means of ICA, we used the GIFT software (http://icatb.sourceforge.net/) (32). Thus, smoothed data of all participants, combining patients and healthy controls, were temporally concatenated into a 4D time series and then decomposed into different temporal dimensions using principal component analysis and constrained to 20 components, to be then analyzed with the Infomax algorithm (33).

ICA was performed 100 times and the results were clustered by GIFT toolbox ICASSO min cluster size of 15 and max of 20 (number of runs) and RandInit and Bootstrap were selected. Finally, there was a back-reconstruction process from the group ICA components estimated to the individual activation values for each participant of the different groups and for each component. This process allowed the estimation of different spatial component maps for each individual in terms of voxel-wise z scores. Then, within the obtained ICA networks in the group of participants, we identified the common resting-state intrinsic connectivity networks by visual inspection, and among them, we selected the networks of interest. Two independent authors (JS, EC) separately reviewed the 20 components from the ICA, and after visual inspection and in accordance to published data (23) identified the DMN, DAN and EXE networks (see **Table 2**).

Functional Connectivity of the Hippocampus and the Networks of Interest

For the resting-state fMRI data, the group-level EXE, DAN and DMN maps were identified using a one-sample t test (including both positive and negative effects) after entering the individual resting-state maps for both patients and controls. In order to define the group level resting-state connectivity map for each network, results were reported using a threshold of $p < 0.05$ with Family-wise error correction for multiple comparisons at whole brain level. The maxima of suprathreshold regions were localized by rendering them onto a normalized T1 structural MNI reference brain.

Then, for each participant, the whole hippocampi and four seed regions (left/right, anterior/posterior) were defined based on hippocampal delineations and, the z-scores across all voxels within the selected region of interest (ROI) were averaged in the different networks at $p < 0.05$ (uncorrected), representing the functional connectivity magnitude between the hippocampal ROI and each network.

The different ROIs were delineated following the same approach described previously (34). Specifically, the segmentation of hippocampi was performed according to the Anatomical Automatic Labeling (AAL) brain atlas (35). The anterior and posterior subdivision was made just posterior of the uncal apex ranged from $y = -2$ to -18 (anterior) and from $y = -24$ to -42 (posterior) in MNI coordinates. Between the anterior and posterior part of the hippocampal division, a gap of 4 mm was left in order to reduce inter-regional effects because of smoothing and registration errors (17).

Statistical Analysis

The statistical analysis was performed with SPSS (v.25, SPSS Inc., Chicago, USA). First, in order to identify those patients that presented cognitive impairment after surgery, we compared the scores of verbal learning task before and after surgery on an

TABLE 2 | Resting-state network functional connectivity for controls and patients ($p < 0.05$ FWE-corrected at whole brain level, cluster extent >20 voxels).

Anatomical region	Cluster size (voxels)	MNI coordinates (x y z)	*t* value
For the default mode network			
L superior medial frontal	6,803	−4 48 30	32.4
R precuneus		12 −52 18	26.4
L Precuneus		−6 −58 16	25.1
L Angular gyrus	2,378	−42 −72 30	25.0
R Angular gyrus	1,815	48 −62 24	19.9
L orbitofrontal	2,912	0 62 −2	17.6
L medial frontal		2 64 10	15.5
L parahipocampal	88	−24 −38 −14	10.3
R middle frontal gyrus	307	24 34 44	10.3
R superior frontal gyrus		26 24 54	9.1
L superior frontal gyrus	245	−18 40 48	8.5
L middle frontal gyrus		−24 26 50	8.1
L middle temporal gyrus	102	−60 0 −22	8.3
For the executive network			
R Supp motor area	11,429	2 12 64	17.8
R Frontal superior medial		10 22 60	17.2
L Frontal superior medial		−6 62 18	16.2
L Superior parietal	76	−8 −58 56	8.6
R Superior parietal		6 −56 54	6.2
R Frontal superior medial	25	18 16 10	7.6
L Caudate	26	−12 0 16	7.2
L middle temporal gyrus		−60 −6 −14	7.6
For the dorsal attention network			
R Superior parietal	7,742	12 −66 58	18.0
L Superior parietal		−8 −60 62	15.6
R Middle frontal gyrus	1,937	24 14 58	11.8
L Supp motor area		−2 2 50	11.7
L Mid-cingulate		2 16 38	11.3
L middle frontal gyrus	444	−24 4 58	10.9
L Supramarginal	80	−58 −28 34	8.4
R Posterior cingulate	81	10 −52 8	8.3

MNI, Montreal Neurological Institute stereotactic space; L, Left, R, right; Supp, Supplementary.

individual level. Specifically, we classified those patients who had a decline of two standard deviation of the normalized score or more in verbal learning as learning-decline, and the patients who showed a decline below two standard deviation or no decline after surgery as learning-intact. In order to define significant decline we applied the two standard deviation cut-off as it is a highly stringent according to previous reports (36).

Second, statistical analysis of groups (learning-decline vs. learning-intact vs. controls) demographics, clinical data including learning scores, and total hippocampal volume was performed. Log linear analysis and ANOVA were used to describe socio-demographic, clinical and hippocampal volume differences. If there was a significant difference among groups, a *post-hoc* univariate analysis using Bonferroni's correction was performed. When comparisons were made between learning-decline and learning-intact groups, T-student and Chi-square tests were performed to assess statistical significance.

Third, to investigate differences in the hippocampal longitudinal axis connectivity according to the neuropsychological performance, a multivariate analysis of variance (MANOVA) with Tukey *post-hoc* test was performed, considering the three groups (controls, learning-decline and learning-intact). First, Box's test was performed to check equality of covariance. Then, overall significance of the MANOVA test was assessed with Pillai's trace. This was followed by univariate analysis with Tukey correction. Finally, in order to corroborate which hippocampal longitudinal axis connectivity with the different networks outcomes could be used to correctly classify the different groups based on the surgical cognitive deficits, a linear discriminant analysis was performed, and a receiver characteristic operator curve (ROC) was configured for the variables showing significant differences on the discriminant function. These analyses were performed for the anterior/posterior division and for the whole hippocampus separately.

RESULTS

After classifying the patients according to the cognitive deficits postoperatively in the verbal learning task, 20 patients were learning-intact and 11 suffered a learning-decline after surgery. Preoperatively, no differences were found between groups on the RAVLT. After surgery, a significant difference was found when comparing controls and learning-intact patients with the patients of the learning-decline group ($F = 7.09$, $p = 0.002$). See **Table 1** for socio-demographic and clinical differences.

Global hippocampal volume differences among the three groups were investigated. Comparing the pre-surgical hippocampal volume, both the learning-intact and learning-decline groups had a decreased hippocampal volume for the to-be resected side when compared with the controls ($F = 9.79$, $p < 0.001$) with no significant differences between the two groups of patients on the *post-hoc* analysis. Also, there were not significant differences in the hippocampal volume among groups on the first-level ANOVA for the contralateral side ($F = 1.97$, $p = 0.154$).

Group-level EXE, DAN and DMN map of patients and controls is shown in **Figure 1**. Regions with a positive coupling corresponded to areas typically reported in the literature as part of the EXE (i.e., dorsal and anterior frontal areas), DAN (i.e., superior parietal and dorsolateral frontal areas) and DMN (i.e., posterior cingulate-precuneus and orbitofrontal regions). We first performed MANOVA among the three groups (learning decline, learning intact, and controls) comparing the connectivity of the whole hippocampus with the DMN, EXE, and DAN networks. Using Pillai's trace, there was a significant difference of the pattern of hippocampal connectivity among the groups [$V = 0.95$, $F_{(12,84)} = 6.3$, $p < 0.001$]. See **Table 3** for details.

For this whole hippocampus analysis, there were differences on the DMN connectivity with both the healthy side [$F_{(2,46)}$

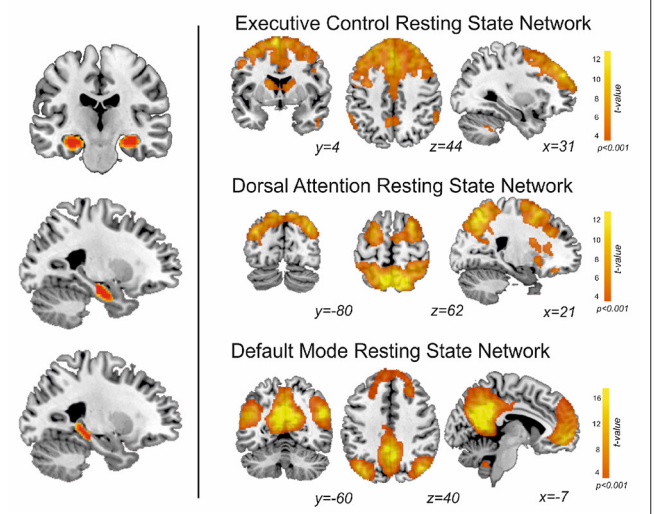

FIGURE 1 | On the left, segmentation of both hippocampi, and division along the anterior-posterior axis. On the right, the resting state networks obtained in this study from patients and controls.

$= 51.2$, $p < 0.001$] and the pathological side [$F_{(2,46)} = 30$, $p < 0.001$], and on the DAN connectivity with the pathological hippocampus [$F_{(2,46)} = 5.54$, $p < 0.007$]. The *post-hoc* analysis revealed a significant decrease in global hippocampal connectivity bilaterally with the DMN for patients (both learning-decline and learning intact) when compared to controls ($p < 0.0001$, Tukey corrected). The *post-hoc* analysis on the pathological hippocampus with the DAN connectivity revealed an increase in connectivity for the patients with learning decline compared to controls ($p = 0.007$, Tukey corrected).

We then performed a MANOVA among the three groups comparing the connectivity of the anterior and posterior hippocampi with the DMN, EXE, and DAN networks. Using Pillai's trace, there was a significant difference of the pattern of hippocampal connectivity among the groups [$V = 0.73$, $F_{(24,72)} = 1.72$, $p = 0.041$]. See **Figure 2** for comparison results, and **Table 3** for connectivity details.

With regard to the anterior connectivity hippocampal pattern, separate univariate tests revealed a significant difference in the pathological side with the DAN [$F_{(2,46)} = 4.75$, $p = 0.013$] and with the EXE [$F_{(2,46)} = 5.65$, $p = 0.006$]. Posterior *post-hoc* analyses showed that these patterns reflect a significant increase in connectivity in the DAN ($p = 0.011$, Tukey corrected) and in the EXE ($p = 0.008$, Tukey corrected) between learning-decline vs. learning-intact patients. Moreover, the non-pathological side showed a significant difference in the anterior connectivity pattern with the DAN [$F_{(2,46)} = 3.90$, $p = 0.027$], in which further post-doc analyses indicated a significant increase of connectivity ($p = 0.022$, Tukey corrected) between learning-decline vs. learning-intact patients.

In contrast, the posterior connectivity hippocampal pattern only showed differences with the DMN, both in the pathological [$F_{(2,46)} = 6.15$, $p = 0.004$], and the non-pathological [$F_{(2,46)} = 3.57$, $p = 0.036$] side. On *post-hoc* analysis, the connectivity of the posterior pathological hippocampus with the DMN was significantly decreased in learning-decline patients when compared with learning-intact patients ($p = 0.029$, Tukey corrected) and controls ($p = 0.003$, Tukey corrected). The connectivity of the posterior non-pathological hippocampus was also significantly decreased in learning-decline patients compared to learning-intact patients ($p = 0.027$, Tukey corrected), but there was no difference with controls. See **Figure 3** for results.

The MANOVA was followed up with a linear discriminant analysis, which provided a multivariate model of how the connectivity measures differentiated the groups. As the discriminant analysis was carried out among three different groups, two different functions were obtained, as per differentiate each group. The functions tried to differentiate the three groups of participants considering a minimum weighted combination of the connectivity measures analyzed.

Using the connectivity of the hippocampus as a whole, the first function significantly discriminated the controls from the patients [Wilkin's lambda $= 0.17$, $X^2 = 77.05$, $p < 0.0001$], but the second function was non-significant [Wilkin's lambda $= 0.850$, $X^2 = 7.06$, $p = 0.216$], so the model was not capable of appropriately distinguishing the groups of patients, thus not identifying the learning-decline patients. On the other hand, for the anterior/posterior division results, the first discriminant function explained 63.3% of the variance, canonical $R^2 = 0.67$, and significantly discriminated the learning-decline patients from the other groups [Wilkin's lambda $=$ 0.37, $X^2 = 40.41$, $p = 0.019$]. Individual discriminant scores for this function correlated significantly with the connectivity between the posterior pathological hippocampus and the DMN (0.67), inversely with the connectivity between the anterior pathological hippocampus and the DAN (-0.63), and finally with the posterior contralateral hippocampus with the DMN (0.47). As such, these three variables demonstrated the most importance in terms of learning-decline group differentiation. The second discriminant function explained the 36.7% remaining variance, canonical $R^2 = 0.570$, but was not significant (Wilkin's lambda $= 0.851$, $X^2 = 7.03$, $p = 0.218$]). See **Figure 4** for details.

Finally, a ROC curve was calculated for the optimal cut-off point to predict verbal learning decline from the three previous connectivity variables which showed significance in the discriminant analysis (posterior bilateral hippocampi to DMN and anterior to-be resected hippocampus to DAN). Also, for the connectivity of the whole hippocampus a ROC curve was calculated, using the bilateral DMN connectivity and the pathological hippocampus to DAN connectivity. The results for the hippocampus as a whole, presented an Area under the Curve (AuC) was 0.63, with an optimal sensitivity of 0.45 and specificity of 0.8, whereas for the anterior/posterior connectivity presented an AuC of 0.84, with

TABLE 3 | Connectivity analysis results for each group.

		L-D	L-I	Controls	*p*
Pathological side					
Anterior hippocampus	DMN	1.2 (2.6)	0.009 (2.2)	1 (2.1)	n.s.
	DAN	1.56 (2.5)	−0.86 (2.3)	−0.37 (1.8)	**<0.05**
	EXE	2.6 (3.5)	−0.61 (2.6)	1.66 (3.4)	n.s.
Posterior hippocampus	DMN	−1.27 (2.1)	0.44 (1.9)	0.96 (1)	**<0.05**
	DAN	0.57 (2.6)	0.41 (2.3)	0.12 (2.5)	n.s.
	EXE	0.13 (3.2)	−0.64 (2.1)	−1.1 (2.3)	n.s.
Whole hippocampus	DMN	−0.38 (2.08)	−0.86 (1.96)	3.64 (2.1)	**<0.05**
	DAN	0.98 (0.98)	0.13 (1.8)	−1.15 (2)	**<0.05**
	EXE	0.1 (2.02)	−0.094 (1.96)	−0.13 (2.4)	n.s.
Healthy side					
Anterior hippocampus	DMN	−0.15 (2.8)	0.29 (2.1)	1.31 (1.8)	**<0.05**
	DAN	2.1 (2.7)	−0.11 (2.2)	1.18 (2.3)	n.s.
	EXE	1.92 (1.8)	0.87 (3.4)	1.4 (3.7)	**<0.05**
Posterior hippocampus	DMN	−0.04 (2.3)	1.61 (1.7)	1 (1.4)	**<0.05**
	DAN	0.74 (2.6)	0.44 (2)	0.81 (2)	n.s.
	EXE	−0.002 (2.7)	−1.05 (2.4)	−0.1 (1.9)	n.s.
Whole hippocampus	DMN	−0.81 (1.09)	0.57 (1.42)	4.84 (2.05)	**<0.05**
	DAN	0.82 (2.54)	0.46 (2.18)	−0.82 (2.05)	n.s.
	EXE	0.71 (2.1)	−0.56 (2.34)	0.12 (1.89)	n.s.

Data represents the z-scores across all values of the hippocampal subregions averaged in the different networks, presented as mean (standard deviation).
L-D, learning decline; L-I, learning intact; DMN, Default Mode Network; DAN, Dorsal Attention Network; EXE, Executive Network. Significance was assessed with a MANOVA test as described previously. The bold values indicate the statistically significant.

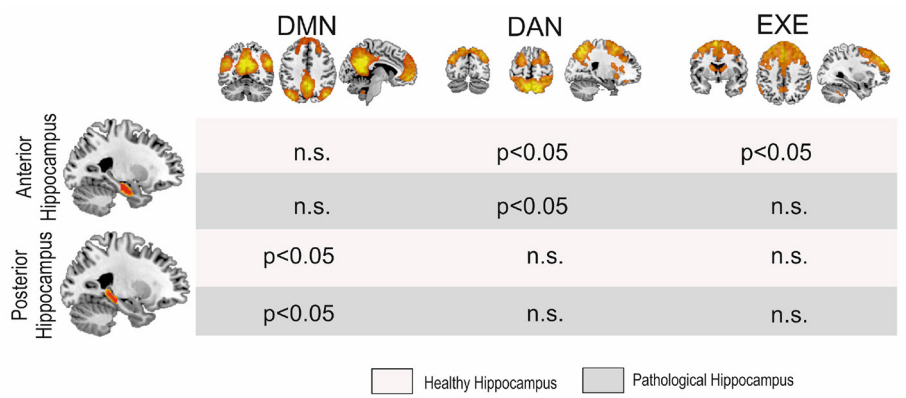

FIGURE 2 | MANOVA results. Significant differences were found among groups in the posterior hippocampi with the DMN and the anterior hippocampi with the DAN and the EXE, although for the latter only with the pathological side. DMN, Default Mode Network; DAN, Dorsal Attentional Network; EXE, Executive Control Network.

an optimal sensitivity of 0.73 and specificity of 0.95 (see **Figure 5**).

DISCUSSION

In this study, we identified functional connectivity signatures related to post-operative verbal learning decline in TLE patients. That is, different connectivity patterns with the RSN along the longitudinal axis of the hippocampus discriminated among patients with and without severe decline in verbal learning after surgery. In particular, we found a pre-operative increase of connectivity of the anterior to-be resected portion of the hippocampus with the DAN, together with a bilateral decrease of connectivity of its posterior portion with the DMN, that appropriately differentiated the group of patients with post-surgery learning-decline from learning-intact patients and controls. In addition, despite the fact that significant changes were found in connectivity among the anterior hippocampi and the EXE network, these changes did not sufficiently discriminate learning-decline patients according to our model.

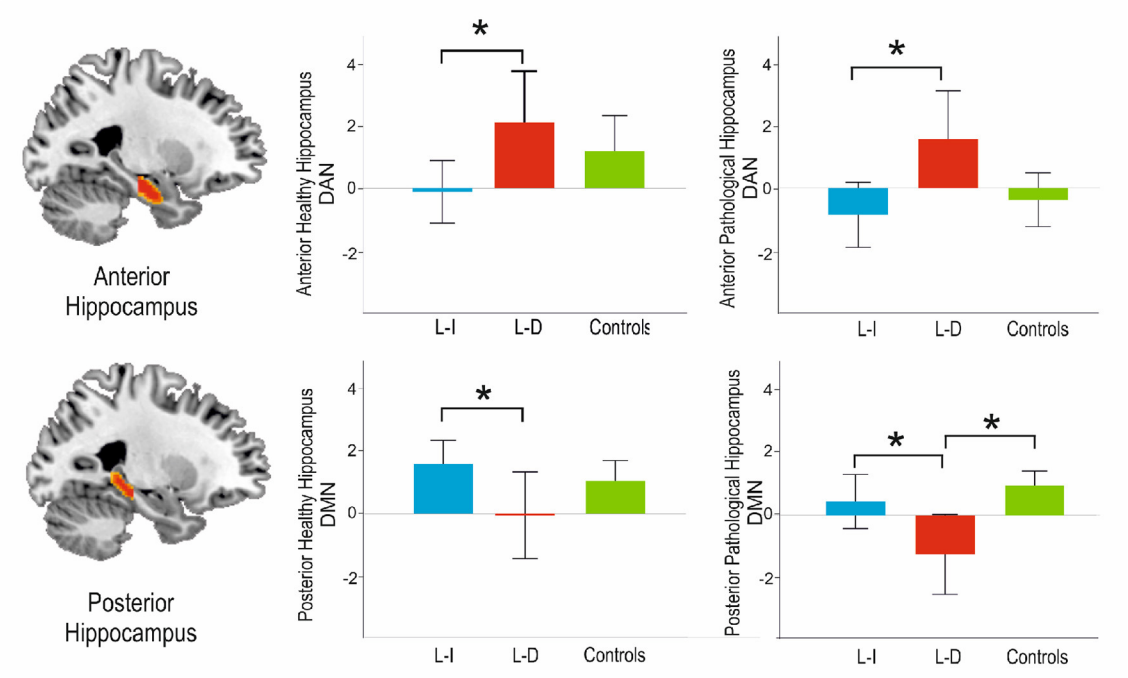

FIGURE 3 | Main results of the *post-hoc* analysis among groups connectivity analyses with the DAN and the DMN along the longitudinal axis of the hippocampus. L-I, Learning Intact; L-D, Learning Decline. *Indicates statistical significance $p < 0.05$.

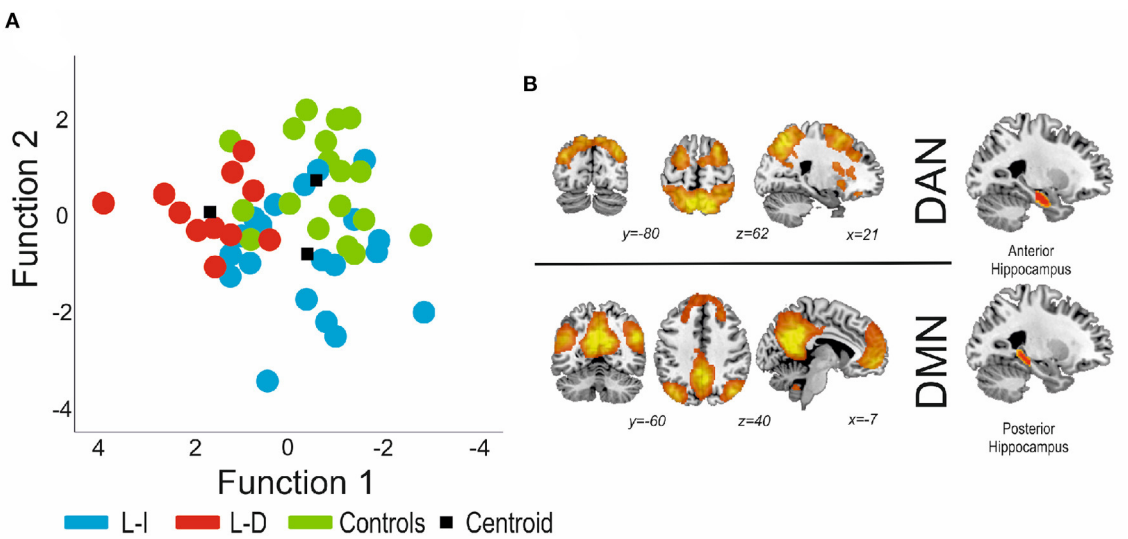

FIGURE 4 | (A) Canonical functions distribution of the discriminant analysis. Function 1 was found to significantly distinguish patients with decline from patients without decline and controls. **(B)** The connectivity variables with higher load were both for the pathological hippocampus (posterior part indicates connectivity with the DMN, and the anterior portion indicates connectivity with the DAN). DMN, Default Mode Network; DAN, Dorsal Attention Network.

Our study revealed two main levels of organization along the long hippocampal axis associated with the verbal learning decline after resection conferring protection against the cognitive impairment after surgery in TLE patients. First of all, according to our data, post-surgery learning-decline patients, learning-intact patients and controls differed in the pattern of the hippocampal formation connectivity with the RSN following an anterior-posterior gradient. Secondly, we found bilateral, up- and

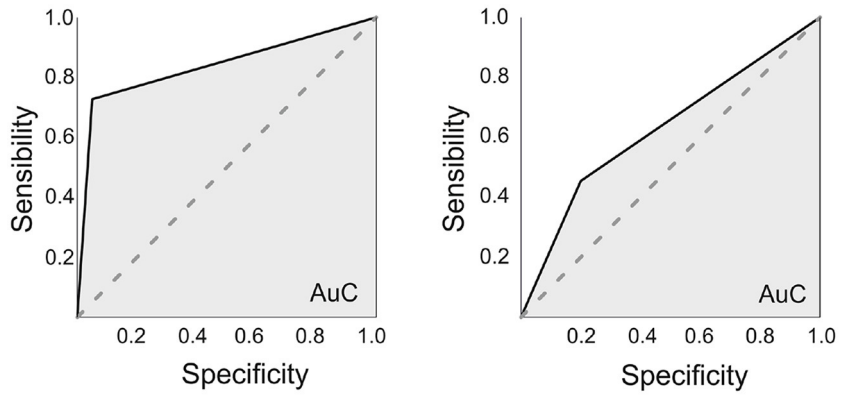

FIGURE 5 | ROC curves. On the left, using the anterior/posterior division of the hippocampi (Connectivity measures for the Posterior bilateral hippocampi-DMN and the anterior to-be resected hippocampus-DAN). The area under the curve (AuC) was of 0.835, with an optimal sensitivity of 0.727 and specificity of 0.95. On the right, using the whole hippocampus (also connectivity measures from the bilateral hippocampi-DMN and the to-be resected hippocampus-DAN). The AuC was 0.63, with an optimal sensitivity of 0.46 and specificity of 0.8.

down-regulations of resting state connectivity, with significant differences in the learning-decline group vs. the learning-intact and control groups. Finally, in our series, investigating the connectivity along the longitudinal axis proved to discriminate learning decline patients from learning intact patients and controls, as compared with the connectivity of the hippocampus as a whole, which was only capable of discriminating patients from controls.

In recent years, the modular paradigm, i.e., postulating specific brain areas as responsible of complex cognitive tasks, has been shifted toward the study of how neural networks influence cognitive processing (37). According to the network paradigm, cognitive functions arise from distributed brain areas comprising multiple distinct, interacting networks. Several large-scale brain networks have been described using resting state functional imaging. It is this connectivity of these RSN with other brain structures such as the hippocampal formation that has proven to be crucial for the maintenance of cognitive tasks (16, 23).

Verbal learning decline has been a consistent finding after temporal lobe surgery (3). Two models were initially proposed as per why some patients showed verbal memory decline after surgery. Namely, these include functional adequacy of the to-be resected hippocampus as sustaining cognitive function, which is thereby lost upon resection, or instead the functional reserve of the contralateral hippocampus (38). However, more recent work switched the attention from the hippocampal model to the connectivity of widespread neuronal networks in order to explain complex cognitive changes that imply different functions (37). In this regard, aligned task-based functional MRI studies using a combination of language and encoding paradigms showed both hippocampal and extra hippocampal activation differences in identifying patients at risk of decline. Thus, the authors postulated that it was the functional adequacy of the network elucidated in the task what was truly relevant for verbal memory decline (39). Taken together, these results, along with evidence on targeting network dysfunction for neurological symptoms (19), pave the way for analysis beyond the lesional level, exploring the cognitive deficits at a network level. Our main hypothesis builds up from this perspective, exploring the role of the disconnection of the hippocampus with the DMN, DAN and EXE, crucial in verbal learning (16, 17).

Our main findings are in line with this network model. Analyzing resting-state connectivity of the hippocampus with the DMN, DAN, and EXE, bilaterally and along its longitudinal axis, significantly discriminated patients at risk of verbal memory decline; a bilateral decrease of the posterior portion of the hippocampi, together with an increased connectivity of the to-be resected hippocampal anterior portion with the DAN was significantly related to verbal learning decline after surgery. Noteworthy, hippocampal connectivity with the RSN followed a functional gradient, that is, the posterior hippocampus with the DMN and the anterior hippocampus with the DAN. Thus, by segregating the connectivity pattern along the axis of the hippocampus, we found significant differences related to the connectivity of the anterior-posterior system (21, 25).

Previous studies evaluating resting state connectivity also reported differences among patients with and without decline (6, 8, 10). In this sense, the definition of different cognitive networks at rest provides an easy and reproducible framework to assess brain functioning. Network studies at rest in TLE patients have shown widespread alterations and correlations to different neuropsychological tasks (10, 13, 40). Using resting-state connectivity, previous studies showed how the connectivity of the hippocampus and the posterior cingulate cortex (PCC), the key hub in the DMN, differed among patients. Relating to their different verbal memory scores, lower strengths of connectivity between the DMN and the hippocampi correlated with poorer performances (41). Furthermore, changes in DMN-hippocampal connectivity may relate with memory loss after surgery (6). Specifically, evaluating the connectivity of the PCC within the DMN to the hippocampus elucidated how patients with episodic memory loss after surgery showed stronger

connectivity with the pathological hippocampus, whereas intact patients showed stronger connectivity with the contralateral hippocampus. In addition, using graph theory measures of areas relevant to cognitive functions and to ictal pathology, Doucet et al. (10) found that neurocognitive deficits after surgery were related mainly to the contralateral hippocampus and widespread bilateral regions, again emphasizing the importance of extratemporal of networks in cognitive functioning.

Nonetheless, the variety of approaches to analyze resting state data, together with the large number of neurocognitive tests reported, limits study comparison. In relation to previously reported studies, we did also find a decrease in contralateral posterior hippocampus-DMN connectivity for the learning-decline patients, but, on the contrary, this group also displayed decreased connectivity between the pathological posterior hippocampus and the DMN. In our approach, we also found that the connectivity of the anterior hippocampus and the DAN was a major discriminant of learning-decline patients. This connectivity has been reported as crucial for encoding processes (22, 42). We hypothesized that an increased connectivity of the anterior pathological hippocampus to the DAN could relate to adaptive changes, relating to the functional adequacy of the to-be resected hippocampus. This pattern of the to-be resected hippocampus, having decreased posterior connectivity to the DMN bilaterally, but increased anterior connectivity to the DAN, could related to a higher vulnerability compared to the learning-intact, who showed the inverse pattern (see **Figure 3**).

Our series consists of patients with different etiologies, with a majority of patients having hippocampal sclerosis (HS). TLE is an heterogeneous disease, even in patients with HS, were heterogeneity in impairment of cognitive domains is found (43). Individual differences are common, likely related to network plasticity (44) against different etiologies and ages of presentation. In our results, different network connectivity of anterior and posterior hippocampus in patients and controls was found, likely related to the different network readjustments in our group of TLE patients, as TLE disrupts large scale cognitive networks (45). Analyzing the differences along the longitudinal axis of the hippocampus could help identify patients at risk of cognitive decline after surgery. In our series, the patients who suffered a verbal learning decline relied on increased connectivity of the anterior pathological hippocampus to the DAN, with decreased connectivity of the posterior hippocampus with the DMN. After resecting the anterior portion of the hippocampus, it is assumed that this connectivity would be disrupted, and therefore the patients suffered a verbal learning impairment.

Our study has several drawbacks. First of all, there was a small number of patients included. Second, we did not consider the extent of resection of the mesial and lateral temporal lobe, which is also associated with memory impairment after surgery (46). Also, most of our sample consists of patients with HS, which could mean that our findings are specific of this condition. Finally, we did not find significant differences between learning-intact and controls, as the discriminant function was not able to differentiate between these groups. Further studies with higher number of participants could be necessary in order to better delineate the learning-intact patients.

On the other hand, applying network analysis along the functional gradient of the hippocampal formation, we report pre-surgical differences among patients with verbal learning decline probably related to individual network plasticity. This finding supports the assumption that RSN—hippocampal connectivity is relevant for cognition, and measuring its integrity could help in the pre-surgical assessment of cognitive risks. However, several other factors should be considered, furthermost the extent of resection, pre-operative memory testing, and verbal lateralization. Nonetheless, the pre-operative connectivity of the mesial temporal area could help predict the risks of verbal learning decline after surgery for TLE. A multivariate prediction system would likely be the best approach in predicting memory deficits after surgery.

CONCLUSION

Our findings support the hypothesis of an anterior-posterior functional division of the hippocampal formation and the cognitive networks. Differences in the pattern of functional connectivity of the DAN and DMN along the longitudinal axis of the hippocampus may have implications on post-operative cognitive deficits and could help identify individually which patients are more at risk of cognitive impairment. Verbal learning impairment remains an important side effect of epilepsy surgery. As a result, personalized counseling based on resting state network connectivity could help in decision making.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Clinical Research Ethics Committee of Bellvitge University Hospital. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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Effects of long-term supplementation of probiotics on cognitive function and emotion in temporal lobe epilepsy

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Cognitive impairment and neuropsychiatric disorders are very common in patients with temporal lobe epilepsy (TLE). These comorbidities complicate the treatment of epilepsy and seriously affect the quality of life. So far, there is still no effective intervention to prevent the development of epilepsy-associated comorbidities. Gut dysbiosis has been recognized to be involved in the pathology of epilepsy development. Modulating gut microbiota by probiotics has shown an antiseizure effect on humans and animals with epilepsy. Whether this treatment strategy has a positive effect on epilepsy-associated comorbidities remains unclear. Therefore, this study aimed to objectively assess the effect of probiotics on cognitive function and neuropsychiatric performance of patients with TLE. Participants enrolled in an epilepsy clinic were randomly assigned to the probiotic and placebo groups. These two groups were treated with probiotics or placebo for 12 weeks, and then the cognitive function and psychological performance of participants were assessed. We enrolled 76 participants in this study, and 70 subjects were finally included in the study (35 in the probiotics group and 35 in the placebo group). Our results showed significant seizure reduction in patients with TLE treated with probiotics. No significant differences were observed on cognitive function (including intelligence and memory) between groups. For neuropsychiatric performances, supplementation of probiotics significantly decreased the Hamilton Anxiety Rating and Depression Scale scores and increased the 89-item Quality of Life in Epilepsy Inventory score in patients with TLE. In conclusion, probiotics have a positive impact on seizures control, and improve anxiety, depression, and quality of life in patients with TLE.

KEYWORDS

probiotics, cognitive function, temporal lobe epilepsy, supplementation, neuropsychiatric disorders

Introduction

Epilepsy is a very common disease in central nervous system affecting approximately 70 million people worldwide (1). Despite the emergence of multiple antiepileptic drugs (AEDs) for the treatment of epilepsy, approximately one in three patients develop drug-resistant epilepsy (DRE) (2). Temporal lobe epilepsy (TLE) is the most common type of epilepsy, which is prone to develop into DRE. Due to abnormal brain discharges originating focally in the temporal lobe and limbic system involving cognitive function and emotion, patients with TLE often suffer from different degrees of cognitive impairment and neuropsychiatric disorders (3–6). These comorbidities severely affect patients' quality of life and burden the family and society (7). Although various drugs targeting a series of comorbidities have emerged, there is still no effective intervention to prevent comorbidity development in patients with epilepsy. Therefore, understanding the pathophysiology of comorbidities associated with epilepsy may help develop therapeutic interventions.

The gut microbiota has gradually been recognized to play an important role in the pathology of epilepsy development (8–10). The gut-brain axis is a bidirectional communication pathway connecting the central nervous and enteric nervous systems, and modulates the neural, immunological, and hormonal pathways to balance the body (11, 12). Gut dysbiosis changes the levels of neurotransmitters, metabolites, and activities in the gut and affects glial function, neuroinflammation, myelination, blood-brain barrier permeability, and neurotransmission, leading to various neurological disorders (8, 13). Recently, a series of studies showed alterations of the gut microbiome in patients with DRE, such as increased relative abundance of Firmicutes and Proteobacteria and decreased Bacteroidetes and Actinobacteria, suggesting that gut dysbiosis may be involved in the pathogenesis of epilepsy (14, 15). Modulation of gut microbiota may be a potential therapeutic strategy for epilepsy. Another study provided evidence that supplementation with probiotics in patients with DRE showed a positive impact on seizure control (16).

Interestingly, a recent study observed that functional gastrointestinal disorders related to microbiota-gut-brain axis dysregulation were significantly associated with the temporal lobe (17). There may be some relationships between microbiota-gut-brain axis dysregulation and neurobehavioral comorbidities, considering the role of the temporal lobe in cognitive function and emotion. However, there is still a lack of relevant research on whether modulating the gut microbiota has a therapeutic effect on epilepsy-associated comorbidities.

Probiotics, regarded as living microorganisms with unknown harmful side effects, provide health benefits to animals and humans by interacting with the intestinal microbiome (18). Modulation of *Bifidobacterium* spp. and *Lactobacillus* spp. were suggested as an effective therapeutic strategy for treatment of DRE (14). BIFICO capsules, containing *Bifidobacterium longum, Lactobacillus acidophilus,* and *Enterococcus faecalis* have been widely used in China for more than 20 years (19). *Enterococcus* spp. increases the colonization of *Bifidobacterium* spp. and provides an anaerobic environment suitable for *Bifidobacterium* spp. *L. acidophilus* produces some growth factors that promote the proliferation of *Bifidobacterium* spp. Combination of these three strains maximizes the prebiotic effect for maintaining gut microbiota balance (20). In this study, we aimed to investigate the effect of BIFICO on cognitive function and emotional symptoms in patients with TLE to provide guidance for treating epilepsy-related comorbidities, thereby improving quality of life.

Methods

Participants and inclusion and exclusion criteria

Subjects were recruited from the epilepsy clinic of the neurology department at Capital Medical University Affiliated Beijing Friendship Hospital from January 2020 to December 2021. This was a double-blind, randomized controlled study with an experimental and a placebo control group. This study was approved by the Human Research Ethics Committee of the Capital Medical University Affiliated Beijing Friendship Hospital. All enrolled patients signed an approved informed consent document after meeting the inclusion criteria.

The inclusion criteria were as follows: (1) aged 50–75 years; (2) TLE diagnosis; (3) seizures that occurred at least twice a month for ≥2 years before entering the study; (4) absence of epilepsy induced by other causes, including encephalitis, stroke, brain tumors, diabetes, or metabolic syndrome; (5) absence of generalized motor seizures, mental motor seizures, or other idiopathic syndromes; (6) no history of neurological or psychiatric disorders and; (7) an ability to read and understand the study documents evaluated by the investigators.

The exclusion criteria were as follows: (1) use of topiramate or phenobarbital, which affect cognition; (2) use of other probiotics or yogurts with live or immune-enhancing supplements within the past 3 months; (3) use of antibiotics or anti-inflammatory therapy within the past 3 months.

We assessed 160 participants for eligibility, and 76 were enrolled in this study according to the inclusion and exclusion criteria (Figure 1). The 76 enrolled participants were equally and randomly assigned to the probiotic and placebo groups. During the 12 weeks of treatment, three participants from the placebo group dropped out of the study; one due to worsened seizures, one due to the long distance involved, and one was lost to follow-up. Additionally, three participants from the probiotic group dropped out in this period; one discontinued due to headaches, and two were lost to follow-up. Therefore, this study eventually

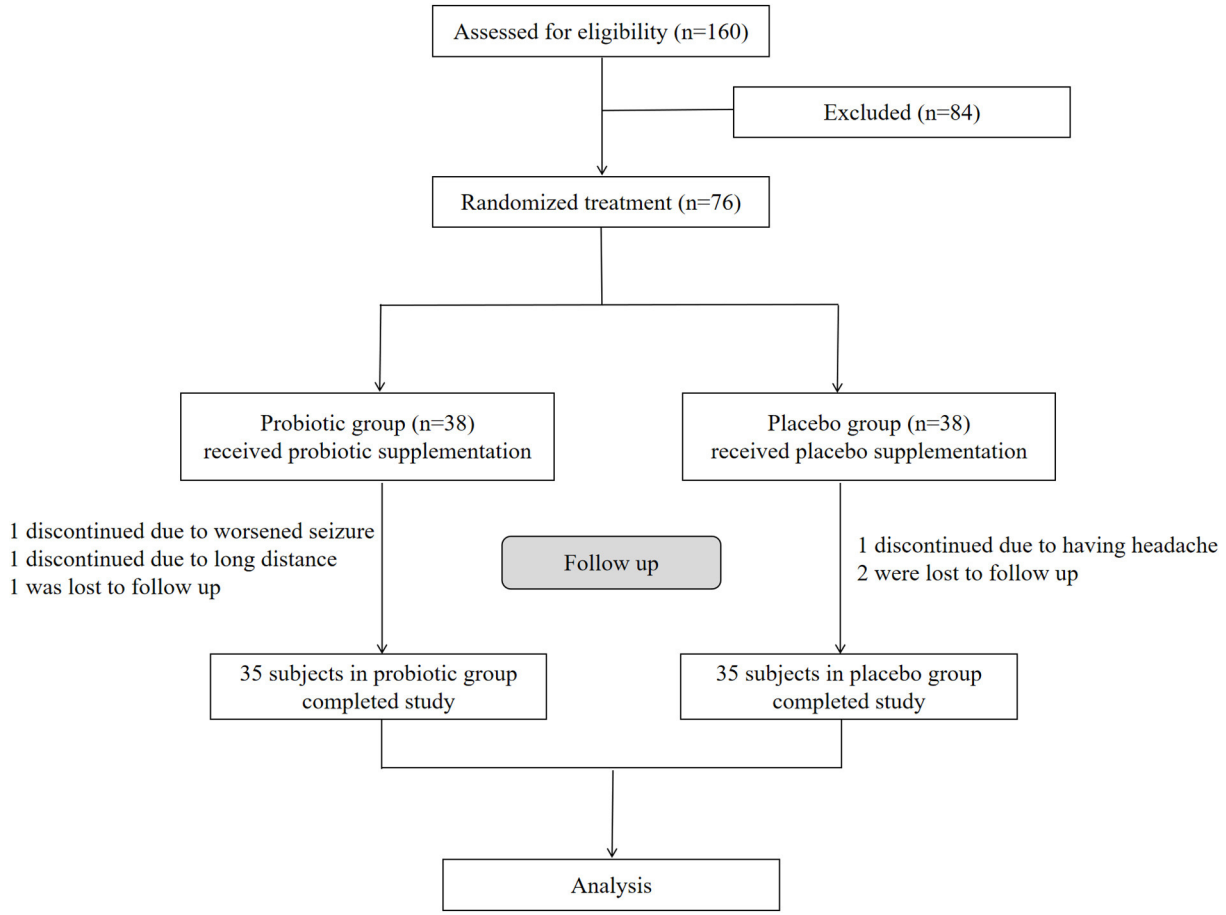

FIGURE 1
Enrollment, randomization, and follow-up of the participants. Seventy six enrolled participants were equally and randomly assigned to the probiotic and placebo groups. After follow-up, 35 participants in probiotic group and 35 in placebo group finally completed the study respectively for analysis.

included 70 participants (35 enrolled in the placebo group and 35 in the probiotic group (Figure 1).

Management and procedures

Participants in the probiotic group were assigned to take two capsules after breakfast and dinner per day for 12 weeks (each capsule contained *B. longum*, *L. acidophilus*, and *E. faecalis*; every living bacterium had $>1 \times 10^7$ colony-forming units). In the placebo group, each capsule contained only 210 mg of starch. The probiotic products and placebos could not be distinguished by package, color, taste, or smell (provided by Shanghai Xinyi Pharmaceutical Co., Ltd., Shanghai, China). Antiseizure treatment for the participants was not changed during the treatment period for either group.

All participants were evaluated for seizure frequency, cognitive function, anxiety, depression, and quality of life, before and after the intervention. All participants, investigators, and researchers were blinded throughout the study period. After the study was completed, all data were used for statistical analysis.

Outcome assessments

Demographic information included sex, age, education years, and body mass index (BMI, kg/m^2). The clinical features included seizure frequency, epileptic history, seizure focus location, and number of AEDs.

The number of episodes in the month before enrollment was regarded as the baseline, and the number of episodes in the third month with probiotic/placebo treatment was regarded as the endpoint. We evaluated the changes of seizure frequency between the baseline and the endpoint.

The Wechsler Adult Intelligence Scale-Fourth Edition (WALS-IV) was used to assess cognitive function (21).

WMS-IV contains ten subtests that contribute to four cognitive spheres: the verbal comprehension index (VCI), perceptual reasoning index (PRI), working memory index (WMI), and processing speed index (PSI). Full-scale IQ (FSIQ) was calculated for all subtests. The scores of the four cognitive spheres and the FSIQ were transformed into an index according to the test manual (M = 100, SD = 15).

The Wechsler Memory Scale-Fourth Edition (WMS-IV) was used to assess participants' memory performance in the probiotic and placebo groups (22). This scale consists of five subtests that produce five indices: the auditory memory index (AMI), visual working memory index (VWMI), visual memory index (VMI), immediate memory index (IMI), and delayed memory index (DMI). According to the test manual, the scores for the five indices were transformed into an index (M = 100, SD = 15).

The Hamilton Depression Scale (HAMD) was used to assess depression severity (23, 24). It contains 24 items, and the total score ranges from 0 to 75. The severity ranges based on the total score for depression were as follows: no depression (<8), mild depression (8–20); moderate depression (21–35); and severe depression (>35).

The Hamilton Anxiety Rating Scale (HAMA) was used to evaluate the level of anxiety (25). This scale consists of 14 symptom-defined items, and the score for each item ranges from zero (not present) to four (severe). Based on the total scores, the severity ranges for anxiety were as follows: absence of anxiety (<7), mild anxiety (7–13), definite anxiety (14–20), obvious anxiety (21–29), and severe anxiety (>29).

The Quality of Life in Epilepsy Inventory (QOLIE)-89 item was used to measure the quality of life. The total score of this inventory provides an estimate of overall health-related quality of life.

Statistical analyses

All data were analyzed using the Statistical Package for Social Sciences (version 21; IBM, Armonk, NY) and GraphPad Prism (version 9; GraphPad Software, San Diego, CA). Shapiro-Wilk test was used to verify the normal distribution of continuous variables. The data of General characteristics and epileptic information were expressed as means and standard deviations and analyzed by using Student's *t*-tests. The categorical data were analyzed by Chi square test and Fisher test. Significant differences were set at a p-value < 0.05. Within-patient contrasts were analyzed by using Mann-Whitney U test that were adjusted by using the Bonferroni correction ($p < 0.005$ for the cognitive measures and $p < 0.008$ for the neuropsychiatric measures). Correlations between variables were assessed using Spearman test.

Results

General characteristics of participants

We assessed 160 participants for eligibility, and 76 were enrolled in this study and randomly assigned to either the probiotic group ($n = 38$) or the placebo group ($n = 38$). Finally, 70 participants completed the study (probiotics, $n = 35$; placebo, $n = 35$), and six subjects dropped out. The patients' demographic and clinical characteristics are shown in Table 1. General characteristics including mean age (probiotics vs. placebo: 60.5 ± 7.0 vs. 60.2 ± 5.8 years), the ratio of females (probiotics vs. placebo: 48.6 vs. 51.4%), years of education (probiotics vs. placebo: 9.3 ± 2.6 vs. 9.1 ± 2.8 years), and BMI (probiotics vs. placebo: 24.9 ± 1.7 vs. 24.6 ± 1.5 kg/m^2) were matched between the two groups with no significant difference ($p > 0.05$). Epileptic information such as epileptic history (probiotics vs. placebo: 10.5 ± 5.1 vs. 9.7 ± 4.9 years), seizure frequency (probiotics vs. placebo: 5.4 ± 1.4 vs. 5.6 ± 1.5 years), focus location (left, probiotics vs. placebo: 45.7 vs. 51.4%), and the number of AEDs did not significantly differ between the two groups ($p > 0.05$).

Effects of probiotics on seizure frequency in patients with TLE

To observe the effect of probiotics on seizure frequency in the participants, the data were classified as no seizure improvement, 1–30% seizure reduction, 30–60% seizure reduction, and 60–100% seizure reduction and analyzed by using chi-square test and Fisher's exact test. As shown in Figure 2, the results were presented as the ratios of participants with no seizure improvement (probiotics vs. placebo: 57.1 vs. 91.4%, $p < 0.05$), 1–30% reduction (probiotics vs. placebo: 31.4 vs. 5.7%, $p < 0.05$), 30–60% seizure reduction (probiotics vs. placebo: 8.57 vs. 2.9%, $p > 0.05$), and 60–100% seizure reduction (probiotics vs. placebo: 2.8 vs. 0%, $p > 0.05$).

Group differences in the WAIS-IV and WMS-IV index scores

On the WAIS-IV, as shown in Table 2, there were no significant differences between the probiotics and placebo groups in the four cognitive indices and FSIQ at baseline. After 3 months of intervention, the scores in probiotic group on PSI, WMI, and FSIQ were observed higher than those in placebo group, but with no significant differences. Similarity, On the WMS-IV, the participants in the probiotic and placebo groups were at the same level on the five indices at baseline (Table 2). At the endpoint, there was no significant differences between

TABLE 1 Demographic and clinical characteristics of participants.

Characteristic	Probiotic group ($n = 35$)	Placebo group ($n = 35$)	*P*-value
Mean age (±SD, years)	60.5 (7.0)	60.2 (5.8)	0.869
Female sex, *n* (%)	17 (48.6%)	18 (51.4%)	0.811
Education (±SD, years)	9.3 (2.6)	9.1 (2.8)	0.696
BMI (±SD, kg/m^2)	24.9 (1.7)	24.6 (1.5)	0.328
Epileptic history (±SD, years)	10.5 (5.1)	9.7 (4.9)	0.465
Seizure frequency (±SD, seizures/month)	5.4 (1.4)	5.6 (1.5)	0.568
Focus location, *n* (%)			0.632
Left	16 (45.7%)	18 (51.4%)	
Right	19 (54.3%)	17 (48.6%)	
Number of AEDs, *n* (%)			0.874
1	9 (25.7 %)	10 (28.6 %)	
2	14 (40 %)	15 (42.9 %)	
3+	12 (34.3 %)	10 (28.5 %)	

Continuous variables were analyzed by Student's t-tests, and categorical variables were assessed by chi-square test. No significant differences between groups.

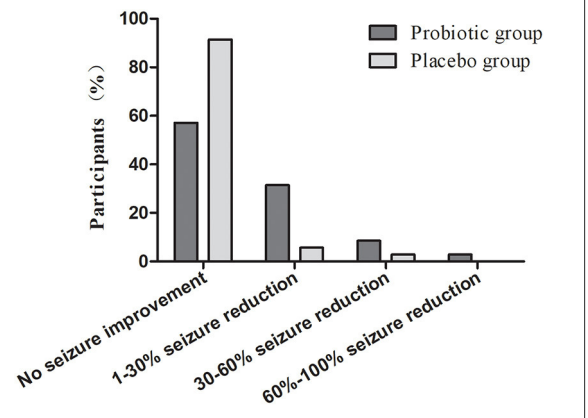

FIGURE 2
Level of seizure improvement in participants with epilepsy after receiving treatment of probiotic and placebo.

the probiotic and placebo groups on AMI, VWMI, VMI, IMI, and DMI.

Group differences in the HAMA, HAMD, and QOLIE-89 scales

Neuropsychological investigations of the participants are shown in Table 3. There were no significant differences between the probiotic and placebo groups in the baseline HAMA, HAMD, and QOLIE-89 scores. After 3 months of intervention, the participants treated with probiotics showed a significant reduction in the HAMA (9.54 ± 5.51 vs. 13.57 ± 6.16, $p = 0.003$) and HAMD (11.83 ± 5.49 vs. 15.23 ± 5.56, $p = 0.006$). There was also an increase in the QOLIE-89 scores (60.29 ± 14.01 vs. 51.91 ± 13.20, $p = 0.006$) compared with those treated with placebo. In addition, an analysis of the effects of seizure reduction on these scales using Spearman test showed that the performance of the participants treated with probiotics had a negative correlation with seizure reduction on HAMA ($r = -0.775$, $p < 0.001$, Figure 3A) and HAMD ($r = -0.696$, $p < 0.001$, Figure 3B) and a positive correlation with seizure reduction on QOLIE-89 ($r = 0.840$, $p < 0.001$, Figure 3C).

Discussion

This study was a prospective examination targeting the effect of probiotics on cognitive function and neuropsychiatric manifestations using comprehensive tests in an adjunctive trial in patients with TLE. This study supports the assertion that supplementation with probiotics effectively increase the antiseizure effect and improve intelligence, memory impairment, anxiety, depression, and low quality of life to a certain extent.

The role of the gut-brain axis in epilepsy has been gradually recognized. A study observed elevated α-diversity in the composition of gut microbiota in patients with DRE, especially those with 4 or more seizures per year (14). Linear discriminant analysis showed increases in the relative abundance of Firmicutes and decreases in Bacteroides in patients with DRE (14). Another study detected fecal microbiota in healthy children and in those with DRE, and found differences in fecal microbial β-diversity between groups instead of α-diversity, and also observed increased relative abundance of Firmicutes and Proteobacteria and decreased abundance of Bacteroidetes and Actinobacteria in infants with DRE (26). Modulation of the gut microbiome may be a potential strategy for treating epilepsy.

TABLE 2 Comparison between groups on WAIS-IV and WMS-IV.

Cognitive function	Baseline				Endpoint			
	Probiotic	Placebo	Z	*P*	Probiotic	Placebo	Z	*P*
WAIS-IV								
VCI	91.37 (15.41)	90.97 (15.61)	−0.065	0.948	92.57 (15.03)	92.14 (15.23)	−0.047	0.962
PRI	84.11 (16.25)	85.03 (16.25)	−0.27	0.787	85.46 (15.83)	85.29 (16.22)	−0.024	0.981
PSI	84.60 (15.77)	82.17 (15.85)	−0.565	0.572	93.77 (15.83)	83.29 (15.91)	−2.716	0.007
WMI	84.26 (15.13)	85.80 (16.67)	−0.406	0.685	95.23 (15.48)	86.57 (17.08)	−2.105	0.035
FSIQ	86.09 (10.30)	85.99 (10.71)	−0.012	0.991	91.76 (9.21)	86.82 (10.54)	−1.915	0.049
WMS-IV								
AMI	83.03 (16.19)	83.29 (16.07)	−0.194	0.846	92.91 (17.19)	83.57 (16.01)	−2.165	0.03
VMI	87.31 (15.56)	85.20 (18.05)	−0.6	0.549	95.54 (15.36)	87.17 (18.25)	−2.016	0.044
VWMI	82.80 (17.34)	82.94 (17.30)	−0.1	0.92	85.09 (18.04)	83.49 (17.69)	−0.388	0.698
IMI	85.14 (15.14)	84.66 (15.73)	−0.147	0.883	94.11 (16.15)	86.17 (15.20)	−2.454	0.014
DMI	85.11 (15.52)	84.11 (15.05)	−0.076	0.939	94.11 (16.53)	86.14 (15.71)	−2.194	0.028

The WAIS-IV and WMS-IV scores are presented in corresponding age-adjusted scaled scores (index scores M = 100, SD = 15). "Z" indicates Mann Whitney U test. Bonferroni correction was applied to determine statistical significance.

TABLE 3 Neuropsychological investigation of participants.

Neuropsychiatric performance	Baseline				Endpoint			
	Probiotic	Placebo	Z	*P*	Probiotic	Placebo	Z	*P*
HAMA	13.83 (6.30)	14.37 (6.34)	−0.453	0.65	9.54 (5.51)	13.57 (6.16)	−2.997	0.003*
HAMD	15.97 (6.03)	16.60 (5.94)	−0.778	0.437	11.83 (5.49)	15.23 (5.56)	−2.743	0.006*
QOLIE-89	48.91 (13.37)	49.71 (13.64)	−0.006	0.995	60.29 (14.01)	51.91 (13.20)	−2.758	0.006*

"Z" indicates Mann Whitney U test. Bonferroni correction was applied to determine statistical significance. *p < 0.008.

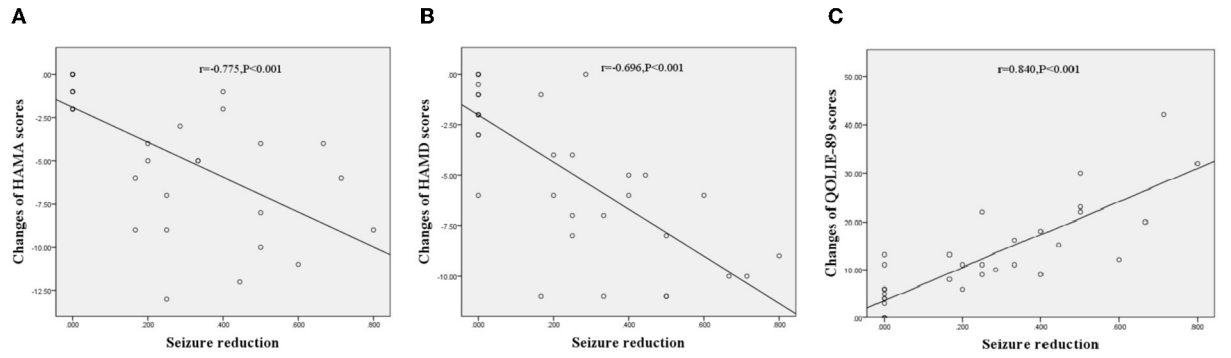

FIGURE 3
Correlations between changes of neuropsychiatric performances treated with probiotics and seizure reduction in TLE patients. **(A)** Correlations between changes of HAMA scores and seizure reduction, $r = -0.775$, $p < 0.001$. **(B)** Correlations between changes of HAMD scores and seizure reduction, $r = -0.696$, $p < 0.001$. **(C)** Correlations between changes of QOLIE scores and seizure reduction, $r = 0.840$, $p < 0.001$.

A case study showed that seizures were controlled in a patient diagnosed with Crohn's disease and epilepsy after receiving fecal microbiota transplantation (27). Ketogenic diets have an antiseizure effect in DRE, and the possible mechanism involves changes in microbiota, microbial interactions, and variance in neurotransmitter and neuroactive peptides levels (15, 28, 29).

Probiotics has been recognized as another promising therapy in epilepsy. In an open-label study, 28.9% of patients with DRE displayed more than 50% seizure reduction following treatment with a cocktail of *L. plantarum, L. acidophilus, L. helveticus, L. casei, B. lactis, Streptococcus salivarius, and L. brevis* (16).

Lactobacillus and *Bifidobacterium* spp. were found to have positive impacts on neurological disorders and psychological diseases (18, 30, 31). A potential therapeutic strategy for epilepsy could be given by modulating levels of *Lactobacillus* and *Bifidobacterium* spp. (14). BIFICO is a cocktail probiotic capsule which consists of *B. longum, L. acidophilus,* and *E. faecalis*. In this study, we observed that supplementation with BIFICO improved seizure frequency in patients with TLE. Current studies have indicated that gut microbiota-derived metabolites and cellular components maintain brain homeostasis. Gut dysbiosis caused by any insult results in disturbance of metabolites and neurotransmitters involved in neural regulation, including 5-hydroxytryptamine, tryptophan, glutamine, gamma-aminobutyric acid (GABA), histamine, short-chain fatty acids, lipopolysaccharides, branched-chain amino acid, bile acids, and catecholamines (13). Abnormalities in these molecules further affect the function of glia, synaptic pruning, myelination, and the blood–brain barrier, which are closely related to seizure susceptibility (10, 13). Supplementation with probiotics may therefore restore brain homeostasis through balancing gut microbiota.

Cognitive impairment is one of several comorbidities with epilepsy. Gut dysbiosis has been found to be involved in cognitive impairment diseases. Bialecka et al. (32) showed that variance in Bacteroidetes and Firmicutes was correlated with mild cognitive impairment, dementia, and Alzheimer's disease (AD), suggesting that alterations in these phyla leading to gut dysbiosis may contribute to cognitive impairment. Probiotics might be an adjustable intervention for cognitive impairment. Razaeiasi et al. (33) observed that treatment with probiotics (*L. acidophilus, B. bifidum,* and *B. longum*) significantly improved spatial learning and memory in rats with AD. Recently, an animal study showed that bacteriotherapy attenuated seizure activity and partially improved spatial learning and memory in pentylenetetrazole-induced kindled rats (34). This may contribute to the improvement in the antioxidant/oxidant ratio and the increased level of GABA stimulated by beneficial bacterial strains in the gut microbiota (34). However, in this human study, supplementation with probiotics was not observed to significantly improve cognitive function. Maybe some individual participants showed obvious improvement on intelligence or memory, but there was no significantly statistical difference. It is necessary to expand the sample size for further analysis. In addition, in animal experiments, timely intervention may effectively prevent the occurrence of cognitive dysfunction in epilepsy, this may be due to the pathology changes in temporal lobe affecting cognition have not yet formed. However, in clinic, pathological changes related to cognitive deficit may have been existed in patients with TLE. At this stage, it is difficult to reverse the pathological changes and therefore may not improve cognitive deficit. More experiments are needed for verification in the future.

Patients with epilepsy have a higher prevalence of anxiety and depression (35). Previous studies have shown that anxiety and depression are risk factors for refractory epilepsy and have suggested a pathological association between neuropsychiatric comorbidities and uncontrolled seizures (36). This special pathological link highlights great challenges in the intervention of epilepsy-associated depression and anxiety. Recently, gut microbiota was found to be involved in the development of neuropsychiatric disorders (31, 37). *Lactobacillus* and *Bifidobacterium* spp. are regarded as psychobiotics with positive impacts in patients with depression and anxiety (38, 39). In this study, we observed that measurements of anxiety, depression, and low quality of life in patients with TLE were significantly improved by supplementation with BIFICO probiotics. These changes were closely related to seizure control. We speculate that there are two possible reasons for this: first, the improvement in anxiety and depression may only contribute to seizure reduction, and second, probiotic-modulated changes in metabolism and neurotransmitters such as dopamine, serotonin, noradrenaline, and GABA may have an effect on TLE pathology (40, 41).

This study had some limitations. First, the sample size was small, and future studies are needed to expand the sample size to verify our results. Second, we conducted our research based on previous studies and did not analyze the metabolic and neurotransmitter effects of probiotics on epilepsy-associated comorbidities. In the future, more studies are needed to study the potential mechanism of the positive impact of probiotics and to build a pathological link between the gut microbiome, epilepsy, and epilepsy-associated comorbidities. Third, clinical seizures were used in this study as the observation index to evaluate whether the improvement of cognitive function and neuropsychological disorders in patients with TLE treated with probiotics were affected by seizure reduction. The impact of interictal activity was not observed in this study, which needs future studies to evaluate and discuss.

Conclusions

In conclusion, the results of this study suggested that BIFICO probiotics have a positive effect on seizure control, anxiety, depression, and quality of life in patients with TLE. This study indicated that BIFICO probiotics was beneficial to TLE patients as adjuvant therapy.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

Ethics statement

The studies involving human participants were reviewed and approved by Human Research Ethics Committee of the Capital Medical University Affiliated Beijing Friendship Hospital. The patients/participants provided their written informed consent to participate in this study.

Author contributions

XW wrote the draft of this article. RM and XL collected and analyzed the data. YZ revised the manuscript and gave the final approval. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Discrepancy between subjective and objective memory change after epilepsy surgery: Relation with seizure outcome and depressive symptoms

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Complaints pertaining to memory functioning are among the most often reported cognitive symptoms in patients with epilepsy. However, research suggests a considerable mismatch between patients' perception of memory functioning and the objective performance as measured with standardized neuropsychological tests. Depressive mood might be an important factor in explaining this discrepancy, though other variables have also occasionally been reported as relevant. There are mixed results as to which role these factors play in determining the overall quality of life of patients with epilepsy. The present study aimed to quantify the mismatch between subjective and objective memory functioning by taking into account the dynamic change of these factors as well as depressive symptoms after epilepsy surgery. Moreover, the influencing factors of subjective and objective memory change were investigated as well as their effects on the overall quality of life. Pre- and postoperative data from 78 patients with focal epilepsy were retrospectively analyzed. The results showed that (1) patients with clinically relevant postoperative depressive symptoms underestimate their actual memory performance; (2) for non-seizure-free patients, a postoperative decrease in depressive symptoms was associated with a tendency to underestimate memory decline; (3) the relationship between objective memory change and quality of life is mediated by the factors subjective memory change and depressive mood. Our data demonstrate a quantitative approximation of a pronounced depression-related negative biased self-perception of memory functioning of roughly 1 to 1.5 standard deviations. Moreover, it seems that when patients are relieved of having recurrent epileptic seizures, they may be less influenced by depressive symptoms when judging their memory

change. Taken together, our study demonstrates the clinical relevance of incorporating subjective measures of memory functioning and mood that go beyond objective memory performance for the interpretation of how changes in memory functioning may affect patients' quality of life after epilepsy surgery.

KEYWORDS

epilepsy surgery, neuropsychology, subjective memory, verbal memory, quality of life, seizures, depressive symptoms

Introduction

In the last decades, neuropsychological research has given us extensive insights into memory functioning in patients with epilepsy (PWE) after epilepsy surgery (1). Effort has also been spent to clarify the subjective perception of memory complaints in PWE. When comparing objective and subjective memory, PWE often tend to misjudge their performances (2–9). It has been suggested that depressed mood (10–13) and psychological distress (4) are factors that might explain this discrepancy, both reflecting an inability to cope with or adjust to the condition (5).

Only a few studies considered these factors and tried to establish an integrative approach to subjective and objective memory functioning as well as psychosocial well-being in PWE over the course of the condition, especially prior to and after epilepsy surgery. In their review, Sherman and colleagues reported on three studies that measured self-reported subjective changes in cognition after epilepsy surgery. Subjective memory loss was evident in 8–20%, whereas memory gains were described in 11–52% of patients. Interestingly, objective memory decline was found in 20–44% of patients (1) suggesting a discrepancy between subjective and objective measures. In line with this, other surgical outcome studies in epilepsy cohorts only found small or no associations at all between subjective and objective memory measures and consistently suggest levels of depression as an indicator for postoperative subjective memory decline (7, 14–17). It has been argued that postoperative mood and subjective memory complaints may also be related to seizure status (14, 18) or medication side effects (14, 15). It may be reasoned that, from a clinical standpoint, subjective memory impacts the psychosocial well-being of PWE more strongly than actual cognitive functioning. Therefore, subjective memory complaints are often disregarded as an 'add-on' in neuropsychological assessment and are often considered as having an inconclusive value in the diagnostic process, because of the many potential factors they interact with.

We argue, however, that there is a substantial need to consider subjective memory complaints pre- and postoperatively since, firstly, it may have decisive value for the individual patient and his or her overall quality of life (QoL). Secondly, it provides the clinician with important additional information not only about emotional well-being but also about its interaction with the cognitive status prior to and after surgery. Thus, for counseling patients for epilepsy surgery and for further planning of psychological treatment options, it seems imperative to disentangle the relationship between subjective and objective memory performance as a function of mood and the implications of it for the overall QoL.

The present study investigated (1) the relationship between mood and pre- to postoperative subjective and objective memory functioning in PWE; (2) how the factor seizure status relates to the change of subjective and objective memory functioning and postoperative mood; (3) the relationship between objective memory change and the overall QoL. We hypothesized that (1) depressed patients do more often report subjective memory deficits (15, 17) and may also overestimate their memory decline from pre- to postoperative assessment. In contrast, there should be no major difference in objective memory performance between depressed and non-depressed patients (5, 19). We further expected that (2) seizure freedom may moderate the association between subjective memory, objective memory, and depressive symptoms (18); patients with ongoing seizures who experience a decrease in depressive symptoms might tend to underestimate their memory decline. Finally, we hypothesized that (3), QoL will not only be affected by depressive mood but by an interplay of this variable with subjective and objective memory scores (5, 20).

Materials and methods

Participants

The study included 78 patients (35 female/43 male) with focal epilepsy who underwent extensive interictal and ictal preoperative video-electroencephalography (EEG) monitoring and epilepsy surgery at the Epilepsy Center Bethel in Bielefeld, Germany (21, 22). Inclusion criteria were: (1) diagnosis of focal epilepsy confirmed by EEG, seizure semiology, and MRI findings during presurgical diagnostics (2), availability of results from standard pre- and postoperative neuropsychological assessment (including self-ratings of cognitive functioning and depressive symptoms), (3) availability of self-ratings of overall QoL at the

TABLE 1 Medical and demographic characteristics of the patient sample.

Variables		$N = 78$
Sex (female)	*n* (%)	35 (44.9)
Age at surgery (years)	M (SD)	39.4 (13.9)
Age at epilepsy onset (years)	M (SD)	16.9 (14.0)
Duration of epilepsy (years until surgery)	M (SD)	22.3 (15.2)
Side of surgery (left)	*n* (%)	28 (35.9)
Site of surgery (non-TLE)	*n* (%)	22 (28.2)
Seizure outcome (Engel 1A/1B)[a]	*n* (%)	47 (61.8)
ASM polytherapy (>1 drug)	*n* (%)	
preoperative		64 (82.1)
postoperative		41 (53.2)
ADM therapy (≥1 drug)	*n* (%)	
preoperative		12 (15.4)
postoperative		14 (18.2)

TLE, temporal lobe epilepsy; M, mean; SD, standard deviation; ASM, anti-seizure medication; ADM, anti-depressive medication.
[a]for three patients, outcome data were carried forward from the 6-months-follow-up assessment; for one patient, outcome was based on a 3.5-year-follow-up assessment.

24-month follow-up between February 2016 and March 2018, (4) age at examination of at least 18 years.

Resection side and type of surgical procedure were specified based on neuroradiological findings and scalp or invasive video-EEG recordings. Seizure freedom was defined as sustained seizure freedom, with or without focal aware seizures, 24 months after surgery (Engel class 1A and 1B) (23). For demographic and clinical characteristics, see Table 1. The study was carried out in compliance with the Code of Ethics of the World Medical Association (Declaration of Helsinki). All patients gave written informed consent to participate in this study. The study protocol was approved by the local ethics committee (University of Bielefeld, Germany, no. 2016-001).

Measures

Objective memory measures

From our standard pre- and postsurgical neuropsychological test battery, we examined test results from the Verbal Learning and Memory Test (VLMT) (24), which is the German adaption of the Rey Auditory Verbal Learning and Memory Test (25). The procedure of this test has previously been described elsewhere (26). Parameters of interest in our study were learning capacity (sum of correctly remembered words throughout trial 1 to trial 5), long-term recall (number of correctly recalled words after a 30-min delay), long-term retention (number of correctly remembered words after five learning trials minus the number of correctly remembered words after a 30-min delay), and long-term recognition (number of correctly recognized words after a 30-min delay). Scores on each variable were transformed into standardized z-scores according to the normative scores of the VLMT. We then calculated the mean of those four z-transformed variables to obtain an overall z-score indicating the overall verbal memory capacity.

Subjective cognitive measures

For the assessment of subjective cognitive functions, patients had to fill in the "Fragebogen zur geistigen Leistungsfähigkeit" (FLEI; Questionnaire for complaints of cognitive disturbances) (27). The FLEI consists of 30 items that assess everyday situations with demands on cognitive functions. Items are divided into three subscales, (i.e., attention, memory, and executive functions). For the purpose of our study, we only included data from the memory subscale (10 items; Cronbach's $\alpha = 0.92$) in our analyses. The participant has to rate each situation in terms of the frequency of experienced disturbances in each of the situations during the past 6 months. Answers are given on a five-point scale (i.e., "never," "rarely," "occasionally," "frequently," and "very frequently"). For an *ad-hoc* translated English version of the FLEI, the reader is referred to (4). The FLEI has been shown to reliably detect subjective cognitive complaints in patients with schizophrenia and depression relative to healthy controls (all three subscales: Cronbach's $\alpha \geq 0.91$, $r_{split-half} \geq 0.87$) (27). As with the objective measures, scores were z-transformed to obtain comparability.

Depressive symptoms

For the assessment of depressive symptoms, patients were asked to fill in the BDI-II (28). The German version of the BDI-II consists of 21 items (Cronbach's $\alpha = 0.93$; $r_{test-retest} = 0.78$) assessing the severity of depressive symptoms (29). It has been thoroughly validated in various patient samples (30). In our study, patients with a BDI-II score below 14 were classified as clinically non-depressed, whereas patients with scores of 14 or higher were classified as having clinically relevant depressive symptoms (29).

Quality of life

For the evaluation of the QoL, patients were asked to fill in the German version of the "Quality of life in epilepsy" questionnaire (QOLIE-31) (31, 32). The QOLIE-31 includes 31 items assessing QoL in PWE. It is an often-used instrument that has been validated in various patient populations and translated into many different languages (33–35). The overall total score-subscale of the QOLIE-31 includes several items on cognition and emotional well-being. As one might expect high intercorrelations between these items and the self-rating

instruments of our study (i.e., BDI-II, FLEI), we did not analyze the overall total score-subscale to prevent redundancy caused by these intercorrelations. Rather, we aimed to detect the specific contributions of objective memory, subjective memory, depression, and QoL, by only analyzing data from the overall QoL scale, which comprises two items asking patients only about their overall QoL ($\alpha = 0.79$; $r_{test-retest} = 0.84$).

Data analysis

To analyze pre- to postoperative change in subjective vs. objective memory in dependance of depressive symptoms, we performed repeated-measures analyses of variance (ANOVA) using depressive symptoms ("depressed" vs. "non-depressed") and measure (objective vs. subjective) as between-subject factors and time (pre vs. post) as within-subject factor. The mean z-score of all VLMT variables was computed to serve as the dependent variable.

To determine the role of seizure frequency in the pre- to postoperative change of subjective and objective memory as well as depressive symptoms, we first computed change scores (i.e., $z\text{-score}_{post}$ minus $z\text{-score}_{pre}$) of the factors subjective and objective memory as well as depressive symptoms. A statistically significant change was based on reliable change indices with 90% confidence intervals. We then calculated the difference between the variables subjective and objective memory change (i.e., subjective minus objective) to get an indicator for the discrepancy between subjective and objective scores. This difference served as a measure of the extent of over- or underestimation of memory change in relation to objective memory change; negative values represent overestimation, whereas positive values represent an underestimation of memory decline, respectively; values near zero represent an adequate estimation of memory change. Subsequently, we calculated Pearson product-moment correlation coefficients between the discrepancy of subjective vs. objective memory change on the one hand, and change of depressive symptoms, on the other hand separately for seizure-free and non-seizure-free patients.

For our third research question, we performed a serial mediation analysis (36, 37) to model the relationship between memory decline and QoL and the mediating influence of the two variables subjective memory change and depressive symptoms. Memory decline was calculated as a dichotomous variable (i.e., decline vs. no decline); a decline of memory functions was defined for change scores ≤ 3 (i.e., raw score_{post} minus $\text{raw score}_{pre} \leq 3$) for the long-term recall of the VLMT corresponding to a statistically significant change based on the 90%-reliable change index (24). Both mediator variables, namely subjective memory change and depressive symptoms represented z-scores. QoL-scores represented raw scores of the overall QoL scale from the QOLIE-31.

Statistical analyses were conducted using SPSS Statistics 25 (IBM, Chicago, USA). The serial multiple mediation analysis was conducted using the PROCESS macro for SPSS, Version 5.5.3 (36, 37). Figures were produced using Matlab R2020a (The Mathworks, Natick, USA) and Microsoft Excel (Microsoft Corporation, Redmond, USA).

Results

In the following sections, we report the results of the above-mentioned analyses with respect to the three research questions outlined in the introduction of this paper. For an overview of means and standard deviations of all outcome variables at pre- and postoperative assessment, the reader is referred to Table 2.

Memory and depressive symptoms

The repeated measures ANOVA for the pre- to postoperative comparisons of patients' memory performance (measure: objective vs. subjective) as well as their postoperative depressive symptoms ("depressed" vs. "non-depressed") revealed significant main effects for both between-subject factors: measure [$F_{(1, 133)} = 11.752$, $p = 0.001$, $eta^2 = 0.081$; objective memory > subjective memory] and depressive symptoms [$F_{(1, 133)} = 20.887$, $p < 0.001$, $eta^2 = 0.136$; non-depressed > depressed]. Furthermore, an interaction effect between these two factors [$F_{(1, 133)} = 14.695$, $p < 0.001$, $eta^2 = 0.099$] was found, showing significantly lower subjective memory scores than objective test results for only the "depressed" patient group but not for the "non-depressed" group. Figure 1 illustrates the pre- to postoperative change of subjective and objective memory scores as a function of depressive symptoms. Furthermore, a significant interaction effect between time and depressive symptoms was found [$F_{(1, 133)} = 8.134$, $p = 0.005$, $eta^2 = 0.058$] indicating a significant decrease in depressive symptoms from pre- to postoperative assessment. The three-way interaction between time, depressive symptoms and measure was not significant [$F_{(1, 133)} = 2.529$, $p = 0.114$]. However, there was a tendency for the "depressed" patient group to indicate a negative change and underestimation of memory functions, while "non-depressed" patients tended to indicate a positive change in memory functioning whilst objective memory scores remained relatively stable.

Memory, depressive symptoms, and seizure status

Seizure-free patients reported higher BDI-II change scores (i.e., decrease of depressive symptoms) as compared to patients with persisting seizures [$t_{(58)} = 2.43$, $p = 0.018$; Figure 2].

TABLE 2 Pre- and postoperative mean outcomes and mean differences in subjective and objective memory performance as well as severity of depressive symptoms and quality of life.

Parameter	Pre			Post			Diff.		
	Total	Depr	Non-depr	Total	Depr	Non-depr	Total	Depr	Non-depr
Subjective memory	-0.47^{63}	-1.29^{13}	-0.26^{50}	−0.27	−1.99	0.18	0.21	−0.70	0.44
(FLEI)	(0.15)	(0.33)	(0.16)	(0.20)	(0.50)	(0.18)	(0.19)	(0.39)	(0.21)
Objective memory	-0.23^{74}	-0.22^{15}	-0.24^{59}	−0.05	−0.30	0.01	0.18	−0.08	0.24
(VLMT)	(0.10)	(0.29)	(0.10)	(0.11)	(.28)	(0.12)	(0.11)	(0.23)	(0.12)
Depressive Symptoms	-0.45^{60}	-1.14^{12}	0.28^{48}	−0.06	−1.87	0.40	0.39	−0.73	0.67
(BDI-II)	(0.15)	(0.35)	(0.15)	(0.14)	(0.15)	(0.09)	(0.15)	(0.32)	(0.15)
Quality of life	n.a.	n.a.	n.a.	72.26^{73}	54.64^{14}	76.44^{59}	n.a.	n.a.	n.a.
(QOLIE-31)				(20.27)	(28.27)	(15.43)			

Means and standard deviations are given in standardized z-scores, except for the QOLIE that is presented in raw scores. Standard deviations appear in parentheses. Superscript numbers represent the observed n of the respective variable and group (applies to pre- as well as post-operative data). FLEI, Fragebogen zur geistigen Leistungsfähigkeit (Questionnaire for complaints of cognitive disturbances); VLMT, Verbal Learning and Memory Test; BDI-II, Beck Depression Inventory; QOLIE, Quality of life in epilepsy questionnaire; Pre, preoperative assessment; Post, postoperative assessment; Diff., difference (Post – Pre); depr, depressed.

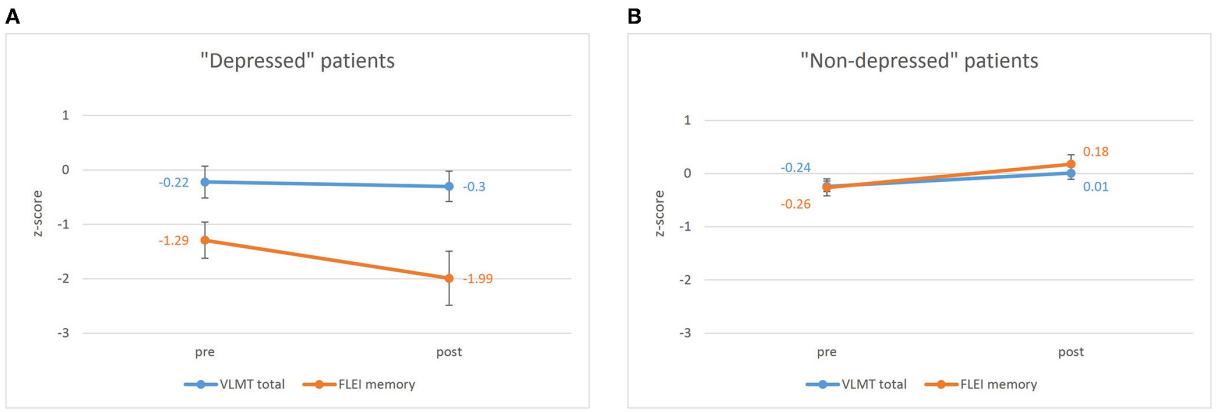

FIGURE 1
Pre- to postoperative results of the VLMT (Verbal Learning and Memory Test) depending on time (pre vs. post) and measure (subjective vs. objective) for **(A)** the "depressed" patient group (BDI-II ≥14) and **(B)** the "non-depressed" patient group (BDI-II <14). Bars represent standard error of mean. pre, preoperative assessment; post, postoperative assessment; VLMT, Verbal Learning, and Memory Test; FLEI, Fragebogen zur Geistigen Leistungsfähigkeit (Questionnaire for complaints of cognitive disturbances).

Pearson product-moment correlation coefficients were computed to assess the relationship between the discrepancy of subjective vs. objective memory change on the one hand and change of depressive symptoms on the other hand. For the whole patient group, there was a moderate significant positive correlation between the two factors discrepancy and change in depressive symptoms ($r = 0.369$, $p = 0.004$) suggesting that a decrease in depressive symptoms was associated with a greater tendency to underestimate memory decline. Splitting the group based on seizure outcome, the positive correlation between the factors discrepancy and change in depressive symptoms was only found in the non-seizure-free group ($r = 0.476$, $p = 0.040$), while for the seizure-free patients, this correlation was not significant ($r = 0.221$, $p = 0.176$, Figure 3).

Memory, depressive symptoms, and quality of life

Results from the serial mediation analysis indicated an indirect relationship between objective memory change and QoL through the two factors subjective memory change and depressive symptoms. In detail, as depicted in Figure 4, a deterioration in objective memory performance from pre- to postoperative assessment was associated with a subjective memory decline ($a1 = -1.24$, $p = 0.012$). This subjective memory decline was subsequently related to an increase in depressive symptoms ($d = 0.29$, $p = 0.004$), which on the other hand was related to a lower QoL ($b2 = 10.51$, $p < 0.001$). A 95% bias-corrected confidence interval based on 10,000 bootstrap samples indicated that this indirect effect through subjective

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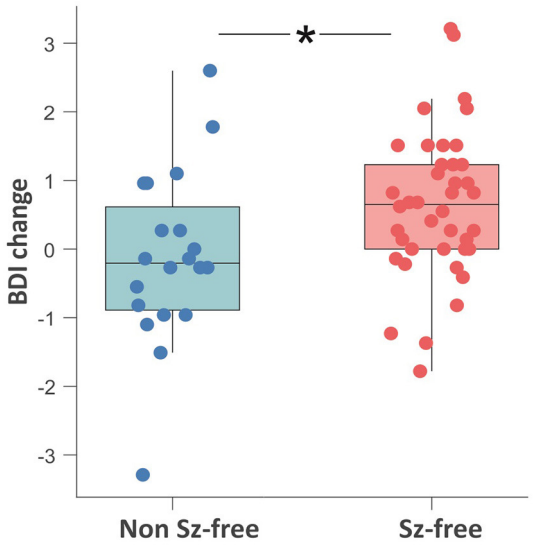


FIGURE 2
Boxplot showing the comparison of the pre- to postoperative change in depressive symptoms between the seizure-free (red) and non-seizure-free patients (blue). Change scores on the y-axis are computed as z-score$_{postoperative}$ minus z-score$_{preoperative}$. Negative values on the y-axis represent a postoperative increase of depressive symptoms. *$p < 0.05$; ns, non-significant; Sz, seizure; BDI, Beck Depression Inventory.

memory change and depressive symptoms (a1 – d – b2 = −3.79) was entirely different from zero (95%- CI: [−9.36, −0.47]). In contrast, the indirect separate effects through both subjective memory change on QoL (a1 – b1), and depressive symptoms on QoL (a2 – b2), respectively, were not different from zero (−8.05 to 2.21 and −19.07 to 1.82, respectively; see Figure 4 for the associated effects). Since the total effect was significant ($c = -16.08$, $p = 0.016$) while the direct effect of objective memory change on QoL ($c' = -3.90$, $p = 0.468$) was non-significant, the relationship between verbal memory change and QoL is fully mediated through subjective memory change and depressive symptoms.

Discussion

As a new approach, the present study investigated the mismatch between subjective and objective memory by taking into account the dynamic change in memory functioning as well as depressive symptoms after epilepsy surgery. Firstly, we found that patients with postoperative clinically relevant depressive symptoms were likely to underestimate memory performance. Secondly, for the postoperatively non-seizure-free patients, a postoperative decrease in depressive symptoms was associated with a tendency to underestimate memory decline. Thirdly, the relationship between objective memory change and QoL was mediated by the perception of memory change and depressive symptoms.

Discrepancy between subjective and objective memory after epilepsy surgery

Despite the relevance of memory functioning in the context of epilepsy surgery, only a few studies investigated the change of subjective vs. objective memory functioning from pre- to postoperative time points. Critically, previous research has predominantly reported correlational evidence for an association between the judgment of memory functioning and emotional well-being or depressive symptoms (16, 17). For the first time, by only analyzing standardized values for both subjective and objective memory scores, we are able to report a quantitative measurement of this discrepancy that goes beyond correlational estimates. It shows a depression-related negative biased self-perception of memory functioning of roughly 1 to 1.5 standard deviations, which appears to be enormous considering the lack of such a bias in "non-depressed" patients. Our finding that clinically "depressed" patients underestimate their memory functions and tend to overestimate memory decline underlines the factor mood as crucial in the judgment of cognitive functioning. This is in line with our first hypothesis and other studies that identified psychopathological variables as crucial in explaining the discrepancy between objective test results and subjective judgments of memory functioning in PWE (4, 10–12). At the same time, this finding clearly indicates that patients' perceptions of memory functioning can be quite accurate in the absence of depressive symptoms. Taken together, our results, for the first time, offer a quantitative approximation of the previously reported mismatch between the perception and the objective performance of memory functioning from pre- to postsurgical assessment in PWE.

Since the nature of a neuropsychological examination in the epilepsy surgery setting (i.e., the assessment of patients at different time points before and after surgical intervention) allows for comparisons of change in specific symptoms or cognitive functions, we can appreciate the data in a dynamic way. By comparing standardized change scores, we found that patients with a decrease in postoperative depressive symptoms tend to underestimate their memory decline. Interestingly, this was only true for the non-seizure-free patients. Therefore, it seems that when patients are relieved of their burdens of having recurrent epileptic seizures, they may be less influenced by depressive symptoms when judging their memory changes in daily life. This matches previous findings demonstrating a subjectively reported relief from anxiety and worries in patients after epilepsy surgery (38, 39). Insofar, our results may be interpreted as a "honeymoon-" or "relief-effect" of seizure freedom and certainly add to the hypothesis that the positive

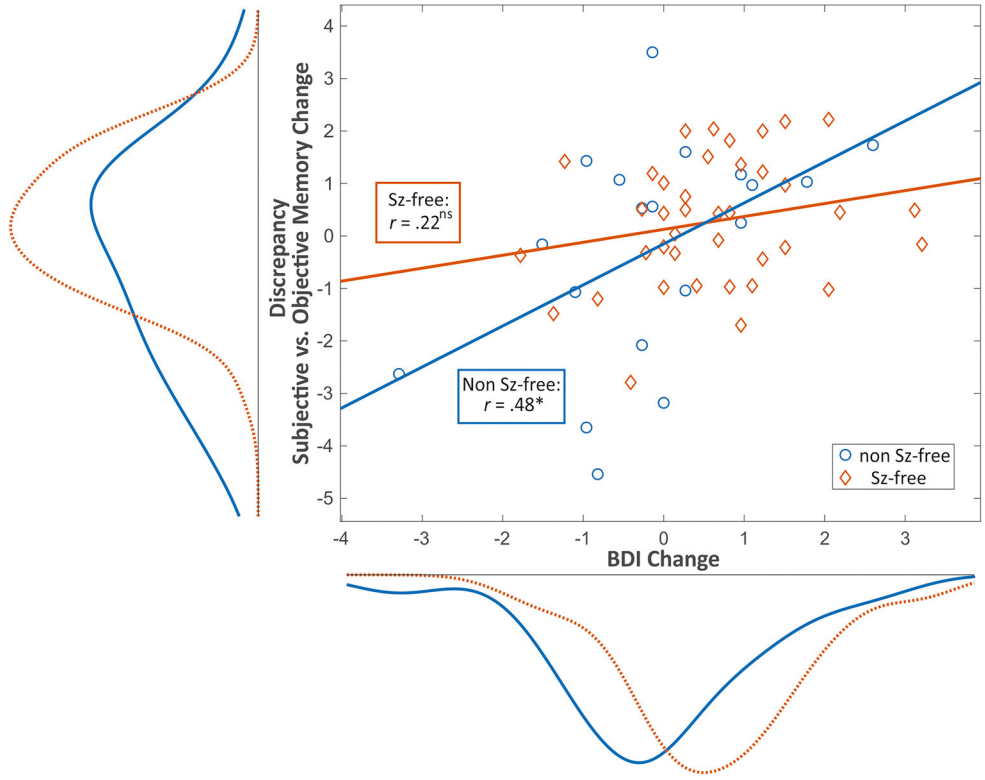
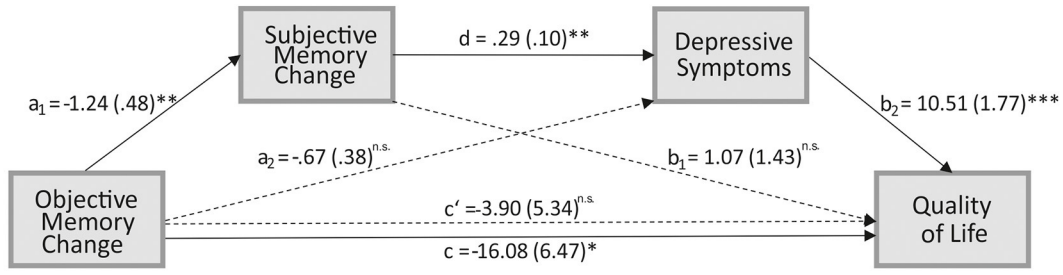


FIGURE 3
Scatter plot showing the relationship between pre- to postoperative change in depressive symptoms (x) and discrepancy (i.e., over-/underestimation) between subjective and objective memory change (y) and the respective distribution curves for seizure-free (red) vs. non-seizure-free patients (blue). Values are given in standardized z-scores. Negative values on the y-axis represent overestimation whereas positive values represent underestimation of memory decline. Values near zero represent an adequate estimation of memory change. Positive values on the x-axis represent a postoperative decrease of depressive mood. *$p < 0.05$; ns, non-significant; Sz, seizure; BDI, Beck Depression Inventory.



Indirect Path	Effect	95%-CI
Obj. Mem. Change → Subj. Mem. Change → Quality of Life	-1.32 (2.58)	[-8.05, 2.21]n.s.
Obj. Mem. Change → Depressive Symptoms → Quality of Life	-7.05 (5.29)	[-19.07, 1.82]n.s.
Obj. Mem. Change → Subj. Mem. Change → Depressive Symptoms → Quality of Life	-3.79 (2.31)	[-9.36,-.47]

FIGURE 4
The serial mediating effect of subjective memory change and mood in the relationship between objective memory change and quality of life (QoL). All presented effects are unstandardized; a_n is the effect of objective memory change on mediators, objective memory decline is coded as 1 and no decline as 0; b_n is the effect of mediators on QoL; c is the total effect of objective memory change on QoL; c' is the direct effect of objective memory change on QoL after controlling for subjective memory change and depressive mood; d is the effect of subjective memory change on mood. *$p < 0.05$, **$p < 0.01$;*** $p < 0.001$; ns, non-significant; obj., objective; subj., subjective; mem., memory.

effects of seizure freedom might even compensate for objective memory deficits (18). Interestingly, these effects are not only present at a specific pre- or postoperative timepoint but seem also to occur as a function of time in the two-year interval from pre- to postoperative assessment. In other words, patients – if seizure-free – do not seem to solely consider their current state when asked about their memory functions but are also able to perceive their change of memory functioning in a specific timeframe.

Furthermore, we found evidence for an association between a change in objective memory functioning and overall QoL after epilepsy surgery. This association was mediated by subjective memory change and depressive symptoms. More specifically, a decline in memory functioning was associated with a decreased QoL, but only when the perception of this decline led to an increase in depressive symptoms. When considering influencing factors of QoL after epilepsy surgery (20), the relationship between neuropsychological variables and QoL has mostly been described as nonexistent (40–42) or was only found in patients with continuing seizures (43). Analyzing different subjective factors in a comprehensive model allows for an explanation or specification of these earlier findings by highlighting the specific mechanisms underlying the association between objective memory change and QoL.

Our results, from a neuropsychological standpoint, suggest that for understanding the relevance of the impact of subjective memory change on the course of the disease and patients' QoL, as well as for the planning of treatment options, it is crucial to consider both, subjective memory functioning and depressive symptoms in the pre- and postsurgical evaluation of PWE. Since we found a strong concordance between subjective and objective memory functioning in the absence of depressive symptoms, the use of standardized memory questionnaires and norm scores (e.g., z-scores), in this case, may serve as a good estimate for memory functioning and, at the same time, allows for comparisons among different measures and time points. Furthermore, the availability of subjective data may help professionals to better recognize individual needs and worries with regard to changes after surgery, eventually leading to more patient-centered care and individualized presurgical counseling and risk assessment (44, 45). We, therefore, recommend the consideration of these variables both when interpreting neuropsychological results, for pre- and postoperative counseling and informing of patients, and for the individualized planning of psychological treatment options after surgery, e.g., guided by evidence-based intervention protocols, such as the HOBSCOTCH program (46). Finally, by considering the change in subjective memory functions and depressive symptoms, we have established a clear impact of objective memory performance on the QoL, which, at least from a patient's perspective, further underlines the clinical significance of self-rating instruments.

Limitations and strengths

First, our patient sample was quite heterogeneous in terms of focus localization and lateralization. This may lead to decreased comparability of our results with results from other studies. On the other hand, we previously found that pertaining to frontal and temporal lobe epilepsies, patients with frontal lobe resections show a verbal memory decline that is, at least in some cases, comparable to memory loss after temporal lobe resections (26). This highlights the relevance of monitoring memory changes in patients with extra temporal lobe epilepsy, especially since this patient group becomes more important in the presurgical setting (22). Moreover, one might argue that subjective memory complaints may also pertain to autobiographical (explicit) memory deficits, or figural memory deficits while the VLMT and FLEI aim to assess verbal memory (generally processed by the left hemisphere). Therefore, it would be interesting to examine whether our results also hold true for memory functions processed more prominently by the right hemisphere. Interestingly, we found that when exploratively including the factor lateralization of epileptic focus in our repeated-measures ANOVA, the main effect of lateralization becomes significant ($p = 0.026$, $eta^2 = 0.038$) while the interaction between lateralization and measure does not become significant ($p = 0.267$). We would like to stress, however that these effects should be interpreted with caution, since including this factor in our analysis led to small subgroups and small statistical power resulting in limited generalizability of these findings. Secondly, it has to be stressed that by assessing depressive symptoms with the BDI-II, we cannot infer a psychiatric comorbidity (29). However, this was not our primary aim; we were more interested in the quantitative degree of subjectively perceived depressive symptoms regardless of formal diagnostic boundaries. It would still be interesting to know whether PWE with high scores on the BDI-II also meet the criteria for a depressive mood disorder since this would have important treatment implications because of the clear effects on other health-related factors. Therefore, future studies should strive to investigate whether our results also apply to PWE that were diagnosed with a depressive mood disorder based on standardized assessments. Thirdly, because of the retrospective design of our study, QoL data was only available from postoperative assessment. Hence, we cannot be sure whether our patient sample experienced an overall improvement in QoL from pre- to postoperative assessment, as has been shown in other studies, at least for seizure-free patients (20, 47). It would have been interesting to compare the amount of QoL change in relation to subjective and objective memory change. Finally, one could argue that, since subjective memory, QoL, and depressive mood are related constructs, assessment of these factors by self-rating instruments (i.e., BDI-II, FLEI, QOLIE-31) would naturally yield high intercorrelations with the effect of causing the above-mentioned results. To

address this issue, we did not analyze the merged scores of all subscales of these instruments, but we intended to measure only the most relevant and specific aspects of these constructs to prevent redundancy and shared variance caused by these intercorrelations (see Sections Objective Memory Measures, Subjective Cognitive Measures, Depressive Symptoms, Quality of Life).

We think that the appreciation of our data in a dynamic timeframe (i.e., *change* of symptoms in contrast to *presence/absence* of symptoms) is a strength of our study and represents a more realistic way of mirroring the course of symptoms or a disease. Our aim was to develop a comprehensive model of memory changes following epilepsy surgery beyond the incorporation of standard factors and analyses. By this means, and by computing standardized change and discrepancy scores, we were able to present data in a flexible and appealing manner to better understand the impact of epilepsy surgery on a subjective level. For example, with this approach, we could quantify the discrepancy between objective and subjective memory, which goes beyond reporting standard correlational data. Future studies should adapt this approach and include other factors of cognitive functioning as well. In addition, future studies should compare patients' progress of (subjective) memory and QoL change with a control group and establish criteria for clinically meaningful change.

Conclusion

Due to changes in epilepsy-related factors, such as fluctuations in seizure frequency, or cognitive changes after epilepsy surgery, epilepsy as a condition is characterized by dynamic changes in symptomatology in the course of the disease. Therefore, it is necessary to study and comprehend these alterations accordingly. By implementing a dynamic approach, we demonstrated a pronounced depression-related discrepancy between subjective and objective memory by showing a negative biased self-perception of memory change after epilepsy surgery. Considering the lack of such a bias in "non-clinically depressed" patients, it reflects the factor mood as crucial in the evaluation of pre- and postoperative cognitive functioning in PWE. Moreover, postoperatively "non-clinically depressed" patients tended to underestimate their memory decline, but only when they were seizure-free after surgery, possibly indicating a relief-effect of seizure freedom. Finally, we found evidence for an association between objective memory change and overall QoL after epilepsy surgery, which was mediated by subjective memory change and depressive symptoms, highlighting mood and memory functioning as crucial factors in the pre- and postsurgical evaluation of PWE. Based on these findings, we plead for the broader consideration of standardized self-rating instruments, even in longitudinal observations of non-surgical patients, with the goal of better interpreting crucial changes in the course of the disease and how these changes may affect patients' QoL.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by the Local Ethics Committee of University of Bielefeld, Germany, no. 2016-001. The patients/participants provided their written informed consent to participate in this study.

Author contributions

FM and PG contributed to conception and design of the study, organized the database, performed the statistical analyses, and generated/ prepared the figures. FM wrote the first draft of the manuscript. PG wrote sections of the manuscript. FM, PG, CB, and MH contributed to manuscript revision, read, and approved the submitted version. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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The correlation of temporal changes of neutrophil-lymphocyte ratio with seizure severity and the following seizure tendency in patients with epilepsy

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Background: Changes in the neutrophil-lymphocyte ratio (NLR) has been reported to be associated with epilepsy. Here we aim to investigate the correlation of temporal changes of NLR level with seizure severity and the follow-up seizure attacks in patients with epilepsy (PWE).

Methods: We performed a retrospective analysis of the laboratory data including leukocyte count and NLR within 24 h of acute seizure and during the follow-up period of 5–14 days after acute seizure (NLR1, NLR2, respectively) in 115 PWE, and 98 healthy individuals were included as controls in this study. The correlation of laboratory data with seizure types, etiology of epilepsy, anti-seizure drugs (ASDs), seizure severity, and the follow-up seizure attacks in PWE was studied.

Results: Leukocyte count ($P < 0.001$) and NLR level ($P < 0.001$) were found significantly different between PWE and controls. On the other hand, a multivariable logistic regression analysis showed that NLR1 level (OR = 2.992, $P = 0.001$) and admission leukocyte (OR = 2.307, $P = 0.002$) were both independently associated with acute epileptic seizures. Especially, higher NLR1 level was significantly associated with status epileptics ($P = 0.013$) and recurrent seizures after admission ($P < 0.001$). Furthermore, the multivariable logistic regression analysis indicated that higher NLR1 was a predictor for the tendency of the following recurrent seizure attacks (OR = 1.144, $P = 0.002$). NLR2 was inversely correlated with ASDs taken ($P = 0.011$). Levels of NLR1 ($r = 0.441$, $P < 0.001$) and NLR2 ($r = 0.241$, $P = 0.009$) were both positively correlated with seizure severity.

Conclusions: Seizures were correlated with the alterations of systemic inflammation reflected by leukocyte and NLR. NLR1 and admission leukocyte were both independently associated with acute epileptic seizures. Higher NLR1 was associated with status epilepticus and independently predicted the

tendency of the following epileptic seizures. NLR2 was significantly associated with ASDs taken. Besides, NLR may be used as a biomarker for seizure severity.

KEYWORDS

neutrophil-lymphocyte ratio, inflammation, seizure severity, recurrent seizure attacks, status epilepticus

Introduction

As a chronic neurological disorder, epilepsy affects over 70 million people in the world and imposes a substantial burden on individuals and society (1). It has been found that seizures can significantly impair the quality of human life and this influence depends on the severity of seizures (2). People with epilepsy visit the emergency room more frequently than the general population, with 22% of children and 13% of adults with epilepsy visiting the emergency room each year (3). Acute seizures have long been known to cause higher early mortality in patients with epilepsy (PWE) (4), though a recent report on a more direct and absolute measure of life expectancy has shown that life expectancy is reduced in symptomatic epilepsies, but, prolonged in other subgroups of epilepsies (5). On the other hand, mortality-related sudden unexpected death in epilepsy (SUDEP) can occur immediately during or after a tonic-clonic seizure (6) and it has been shown to be related to the severity of epilepsy (7, 8). Hence, it is necessary to identify possible biomarkers to help predict or mark the development of epilepsy as well as the associated seizure severity for early intervention.

Multiple evidences have indicated the correlation between neuroinflammation and epilepsy. Epileptic seizures provoke neuroinflammation which reciprocally facilitates epileptic seizures (9–12). For example, seizures trigger an increase in pro-inflammatory mediators including COX-2, IL-1β, IL-6, HMGB1, TNF-α and chemokines, which in turn exacerbate epilepsy development (13). Furthermore, neuroinflammation has been shown to contribute to the onset and recurrence of epileptic seizures by lowering its threshold (14). The association of systemic inflammation with epilepsy has also been established (15, 16). Additionally, anti-inflammatory therapy has seizure-suppressing effect (17), such as glucocorticosteroids, an inflammation inhibitor, has significant effect in treating epilepsy of non-inflammatory etiology (18).

Neutrophil–lymphocyte ratio (NLR), which is calculated directly from the complete blood cell count, has been established as a biomarker for systemic inflammation. In previous studies, a relationship between increased level of NLR and central nervous system (CNS) diseases, such as neurodegenerative and cerebrovascular diseases, has been established (19–22). The association of NLR level with epilepsy has been investigated in several studies (23, 24). However, it is still unclear of the relationship of NLR with either the severity of acute seizure or the recurrence of follow-up seizures in PWE. In this study, the association of NLR with the acute seizure severity and the follow-up seizure attacks in PWE will be studied. Besides, the association of temporal changes of NLR level after an acute seizure attack with seizure type, etiology of epilepsy, anti-seizure drugs (ASDs) and status epilepticus (SE) will also be investigated in this study.

Materials and methods

Study population

We conducted a retrospective cohort study of PWE in the Department of Neurology, The First Affiliated Hospital of Anhui Medical University from November 2017 to March 2022. This retrospective study was approved by the institutional review board. All PWE were diagnosed according to the International League Against Epilepsy (ILAE) guideline (25). The inclusion and exclusion criteria were shown in Table 1. The inclusion criteria for PWE were: ≥16 years in age, admission for an acute epileptic seizure and availability of a complete blood count with laboratory data at admission (within 24 h of acute seizure) and during the follow-up period of 5–14 days after admission. We excluded patients who had a concurrent or recent infection

TABLE 1 The inclusion and exclusion criteria of patients with epilepsy.

Inclusion criteria	Exclusion criteria
• Diagnosis of epilepsy according to the ILAE criteria; • Age ≥16 years; • Admission for an acute epileptic seizure; • Availability of a complete blood count with differential laboratory data at admission and during follow-up period of 5–14 days after admission.	• A concurrent or recent infection; • An acute or subacute traumatic brain injury; • An acute or subacute of cerebrovascular disease; • A recent myocardial infarction; • Hematological disease; • Malignancy; • Severe liver or kidney disease; • Pregnant; • Receiving immunomodulatory therapy.

PWE, patients with epilepsy.

which was active within 2 weeks before admission including local and systemic infection, such as lung infection, urinary tract infection, and endocarditis. At the same time, acute or subacute traumatic brain injury, acute or subacute cerebrovascular disease within 2 weeks, recent myocardial infarction within 3 months before the study, hematologic diseases, malignancy, and severe liver and kidney diseases were excluded. In addition, patients who were pregnant or receiving immunomodulatory therapy were not included. SE was defined as a seizure lasting ≥30 min, generalized convulsive SE was defined as (1) seizure duration ≥5 min or two or (2) more discrete seizures with incomplete recovery of consciousness between seizures (25). Epilepsy diagnosed for the first time with first prescription of ASDs on admission was defined as newly diagnosed epilepsy (26). Finally, 115 PWE were included in the study.

A total of 500 healthy individuals were selected based on previous health examination records, among which 98 cases were randomly selected as controls. The control group and PWE group were matched in gender and age.

Data collection and outcome measures

The laboratory data and image information of all patients at admission were retrospectively collected based on the hospital electronic medical record system. Complete blood cell count and leukocyte classification laboratory data were recorded within 24 h of the acute seizure and during a follow-up period of 5–14 days after admission. Blood pressure, C-reactive protein (CRP), liver and kidney function, blood glucose, electrolytes and lipids, electroencephalogram (EEG) and brain magnetic resonance imaging (MRI) were measured in all patients. All brain MRI data were interpreted by experienced neurologists and neuroradiologists who were unaware of the clinical factors of the patients. Duration of illness, epileptic seizure types, the follow-up seizure attacks after admission and the use of ASDs before admission were also collected for all patients. The National Hospital Seizure Severity Scale (NHS3) which contains seven seizure-related factors on a score of 1 to 27 was used to assess the severity of epilepsy (27, 28).

In this study, we reviewed baseline laboratory and clinical factors in patients and healthy controls, including gender, age, systolic blood pressure (SBP), diastolic blood pressure (DBP), albumin, triglyceride (TG) and total cholesterol (TC). A complete blood cell count including total leukocyte count, neutrophil count, and lymphocyte count was collected within 24 h of the acute seizure and during a follow-up period of 5–14 days after admission. As there was no follow-up period in the control group, single laboratory data was included in this study. NLR was calculated by dividing the absolute number of neutrophil by the absolute number of lymphocyte at each time point. NLR at admission within 24 h of an acute seizure was defined as NLR1 and NLR during the follow-up period of 5–14 days after admission defined as NLR2. If more than one laboratory data was available for the NLR, the maximum value was recorded.

Statistical analyses

All the data were statistically analyzed with SPSS 25.0 statistical software. Continuous variables, if normally distributed, were presented as means ± standard deviation. For non-normally distributed variables, the median of the interquartile range was used for analysis. The independent sample *t*-test and Pearson's correlation were used for comparison of means, the chi-square test was performed for comparison of categorical variables. Mann Whitney U test and Spearman's correlation were used for non-normally distributed data. Univariate and multivariate logistic regression analyses were used to exclude confounding factors and predict risk factors. A receiver operating characteristic (ROC) curve and the area under the curve (AUC) were used to determine the sensitivity and specificity of the NLR diagnostic test. In all analyses, $P<0.05$ was defined as statistically significant.

TABLE 2 Demographic and laboratory characteristics of patients with epilepsy and controls.

	Epilepsy ($n = 115$)	Control ($n = 98$)	*P* value
Gender(M / F)	68/47	53/45	0.540
Age (year)	45 ± 17(16–81)	45 ± 15(18–77)	0.969
SBP (mmHg)	126.17 ± 21.56	124.26 ± 14.03	0.910
DBP (mmHg)	77.78 ± 14.41	74.73 ± 9.36	0.134
Albumin (g/L)	40.80(35.50–44.00)	45.85(44.35–47.93)	<0.001
TG (mmol/L)	1.04(0.79–1.47)	1.27(0.87–1.81)	0.084
TC (mmol/L)	3.97(3.52–4.79)	4.79(4.15–5.35)	<0.001
Leukocyte1(*10^9/L)	9.25(6.81–13.10)	5.76(4.81–7.04)	<0.001
Neutrophil1(*10^9/L)	6.86(4.23–9.93)	3.18(2.45–4.00)	<0.001
Lymphocyte1(*10^9/L)	1.47 (0.96–2.04)	2.02(1.66–2.49)	<0.001
NLR1	4.41 (2.42–8.65)	1.53(1.23–2.00)	<0.001
Leukocyte2 (*10^9/L)	6.50(5.31–7.71)	5.76(4.81–7.04)	<0.001
Neutrophil2(*10^9/L)	4.11 (2.86–5.07)	3.18(2.45–4.00)	<0.001
Lymphocyte2(*10^9/L)	1.54 (1.21–1.99)	2.02(1.66–2.49)	<0.001
NLR2	2.40 (1.79–3.44)	1.53(1.23–2.00)	<0.001
Disease duration (year)	3.89 ± 8.22(0–40)	–	–
NHS3 score	11.15 ± 3.75(1–19)	–	–

M, male; F, female; SBP, systolic blood pressure; DBP, diastolic blood pressure; TG, triglyceride; TC, total cholesterol; Leukocyte1, admission leukocyte count; Neutrophil1, admission neutrophil count; Lymphocyte1, admission lymphocyte count; Leukocyte2, follow–up leukocyte count; Neutrophil2, follow–up neutrophil count; Lymphocyte1, follow–up lymphocyte count; NLR, neutrophil–lymphocyte ratio.

Results

The main demographic and laboratory characteristics of the PWE group and healthy controls

A total of 115 patients (mean age 45 ± 17 years, age range 16–81 years, 67 male, 48 female) and 98 healthy controls (mean age 45 ± 15 years, age range 18–77 years, 53 male, 45 female) were included in this study. The main demographic and laboratory characteristics of the patients and healthy controls were presented in Table 2. Compared with healthy controls, admission leukocyte count ($P < 0.001$), follow-up leukocyte count ($P < 0.001$), admission neutrophil count ($P < 0.001$), follow-up neutrophil count ($P < 0.001$), levels of NLR1 ($P < 0.001$), NLR2($P < 0.001$) and TC ($P < 0.001$) were all significantly increased, nevertheless, admission lymphocyte count ($P < 0.001$), follow-up lymphocyte count ($P < 0.001$) and albumin level ($P < 0.001$) significantly decreased in PWE group. No significant differences in gender, age, SBP, DBP and TG were found between two groups.

Neutrophil and lymphocyte counts were not included in multi-factor analysis due to the collinearity of NLR with neutrophil and lymphocyte counts. Therefore, univariate analysis showed that the factors associated with seizures were admission leukocyte count, NLR1 level, follow-up leukocyte count, NLR2 level, TC and albumin levels. Multivariate logistic regression analysis (Table 3) showed that NLR1 level (OR = 2.992, $P = 0.001$) was independently associated with acute seizures after adjusting the levels of admission leukocyte, follow-up leukocyte, NLR1, NLR2, TC and albumin. Meanwhile, admission leukocyte count (OR = 2.307, $P = 0.002$) and albumin level (OR = 0.685, $P < 0.001$) were also significantly associated with acute seizures.

Temporal changes of neutrophil-lymphocyte laboratory data for PWE

In PWE group, the numbers of leukocyte ($P < 0.001$) and absolute neutrophil ($P < 0.001$) and the level of NLR ($P < 0.001$)

TABLE 3 Univariate and multivariate logistic analyses of parameters associated with acute epileptic seizure in patients with epilepsy.

	Crude OR (95% CI)	*P* value	Adjusted OR (95% CI)	*P* value
Leukocyte1	0.559(0.466–0.671)	<0.001	2.307(1.356–3.923)	0.002
NLR1	0.244(0.153–0.388)	<0.001	2.992(1.579–5.669)	0.001
Leukocyte2	0.772(0.653–0.913)	0.002	0.652 (0.399–1.064)	0.087
NLR2	0.226(0.137–0.372)	<0.001	1.395 (0.773–2.517)	0.269
TC	1.763(1.3–2.392)	<0.001	0.700 (0.424–1.155)	0.162
Albumin	1.398(1.264–1.546)	<0.001	0.685 (0.576–0.814)	<0.001

Leukocyte1, admission leukocyte count; Leukocyte2, follow–up leukocyte count; NLR, neutrophil–lymphocyte ratio; TC, total cholesterol.

TABLE 4 Comparisons of laboratory and clinical characteristics between admission and follow–up period in patients with epilepsy.

		Admission (within 24 h)	Follow–up (5–14 days)	*P* value
Leukocyte(*10^9/L)		9.25(6.81–13.10)	6.50(5.31–7.71)	<0.001
Neutrophil(*10^9/L)		6.64(4.23–9.84)	4.11 (2.86–5.07)	<0.001
Lymphocyte(*10^9/L)		1.47(0.96–2.04)	1.54 (1.21–1.99)	0.201
NLR		4.41 (2.42–8.65)	2.40 (1.79–3.44)	<0.001
The follow–up seizure attacks	Recurrent seizures	–	33(28.7%)	–
	No seizures	–	82(71.3%)	–
Classification of epilepsy	Newly diagnosed	56(48.7%)	56(48.7%)	–
	Chronic epilepsy	59(51.3%)	59(51.3%)	–
Brain MRI	Abnormal	68(59.1%)	68(59.1%)	–
	Normal	47(40.9%)	47(40.9%)	–
Anti–seizure drugs	Yes	48(41.7%)	48(41.7%)	–
	No	67(58.3%)	67(58.3%)	–
Type of epilepsy	Self–limited seizures	64(55.7%)	64(55.7%)	–
	Status epilepticus	51(44.3%)	51(44.3%)	–

NLR, neutrophil–lymphocyte ratio.

were significantly higher within 24 h of acute seizure compared with the follow-up period of 5–14 days after admission (Table 4).

Analyses of possible predictors of recurrent seizure attacks after admission

During the follow-up period of 5–14 days after admission, 33 patients had recurrent seizures before NLR2 level were obtained, and 82 patients had no recurrent seizures (Table 4). Univariate analysis used to identify factors influencing the recurrent seizures after admission showed that higher level of NLR1 and status epilepticus were both associated with the recurrent seizures after admission (Table 5). Furthermore, multivariate logistic regression analysis indicated that NLR1 level was an independent predictor of following recurrent seizures after severe epileptic seizure attacks (OR = 1.144, P = 0.002) (Table 6). We further investigated the predictive value of NLR1 for recurrent seizures in these patients after acute seizures. ROC analysis of NLR1 level showed that the best cut-off value was 5.56, AUC was 0.717 (95% CI: 0.611–0.823, $P < 0.001$), and the sensitivity and specificity were 73 and 71% respectively (Figure 1), indicating that the level of NLR1 could predict the risk of recurrent seizures after admission.

TABLE 5 Comparison of baseline characteristics between patients with and without following recurrent seizures after admission.

	Patients with following recurrent seizures ($n = 33$)	Patients without following recurrent seizures ($n = 82$)	P value
Gender (men)	21	46	0.458
Age (year)	45.27 ± 17.15	45.33 ± 17.60	0.988
Leukocyte 1(*$10^9/L$)	10.04(7.20–13.71)	8.49(6.19–12.07)	0.101
NLR1	8.01(3.77–14.42)	3.72(2.07–6.70)	0.000
SBP (mmHg)	126.24 ± 18.25	126.15 ± 22.86	0.670
DBP (mmHg)	78.03 ± 14.32	77.68 ± 14.54	0.601
Albumin (g/L)	41.20(35.15–43.80)	40.80(35.63–44.10)	0.819
TG (mmol/L)	1.22(0.84–1.62)	1.00(0.78–1.39)	0.131
TC (mmol/L)	4.31(3.54–5.08)	3.85(3.52–4.59)	0.246
NHS3	11.97 ± 4.03	10.82 ± 3.61	0.137
Chronic epilepsy	15	44	0.426
Abnormal brain MRI	20	48	0.838
SE	20	31	0.026
ASDs taken	14	34	0.925
Disease duration (≥5 years)	10	13	0.080

Leukocyte1, admission leukocyte count; NLR, neutrophil–lymphocyte ratio; SBP, systolic blood pressure; DBP, diastolic blood pressure; TG, triglyceride; TC, total cholesterol; SE, status epilepticus.

The correlation between NLR1 and NLR2 in PWE

Sixteen out of 115 patients (13.9%) had focal seizures and 9 of the 16 patients also had secondary bilateral tonic-clonic seizures. Ninety nine out of 115 (86.1%) had generalized seizures and 84 of the 99 patients had generalized tonic-clonic seizures. Out of the 115 patients, 56 had newly diagnosed epilepsy and 59 had chronic epilepsy. Abnormal brain MRI was found in 68 patients and normal brain MRI found in 47 patients. Fifty one patients were admitted because of SE and the remaining 64 patients admitted because of self-limited seizures. Before admission, 48 patients were on treatment with ASDs and the others were not on treatment with ASDs. Regarding to the NLR levels measured within 24 h of acute seizure and during the follow-up period of 5–14 days after admission, no difference was found in NLR level between newly diagnosed epilepsy and chronic epilepsy (NLR1: $P = 0.546$, NLR2: $P = 0.474$) (Figure 2A, Table 4). Also, no significant difference in NLR level was found between patients with and without abnormal brain MRI (NLR1: $P = 0.712$, NLR2: $P = 0.833$) (Figure 2B, Table 4). To investigate the effect of different focal lesions on the level of NLR, 68 patients with abnormal brain MRI were divided into subgroups secondary to cerebrovascular disease (CD) (31 cases), traumatic brain injury (TBI) (14 cases), hippocampal sclerosis (HS) (6 cases), focal cortical dysplasia (FCD) (5 cases), and subgroup secondary to other abnormalities (12 cases). No significant difference in NLR level was found among the five categories of patients with focal seizures (NLR1: $P = 0.368$, NLR2: $P = 0.536$) (Figure 2C). As for the treatment with ASDs before admission, 26 cases were treated with monotherapy and 22 treated with combined drugs. As for types of ASDs, sodium valproate was used in 25 cases, carbamazepine in 12

TABLE 6 Univariate and multivariate logistic analyses of possible predictors for the following recurrent seizure attacks after admission.

	Crude OR (95% CI)	P value	Adjusted OR (95% CI)	P value
NLR1	1.156(1.066–1.253)	0.000	1.144(1.053–1.243)	0.002
SE	2.531(1.105–5.797)	0.028	0.523(0.216–1.266)	0.151

NLR, neutrophil–lymphocyte ratio; SE, status epilepticus.

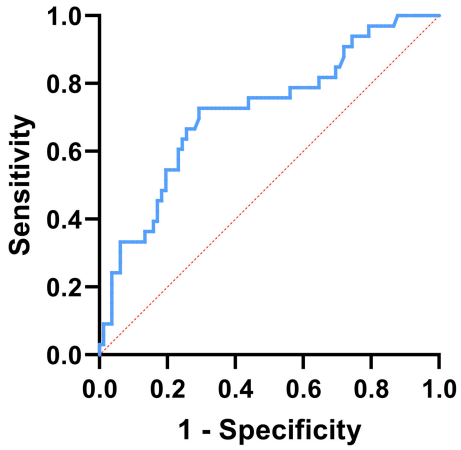

FIGURE 1
ROC curve analysis of the prediction for the following recurrent seizure attacks based on NLR1. The best cut-off value of NLR1 was 5.56, area under the curve (AUC) = 0.717, $P < 0.001$, 95% CI: 0.611–0.823, sensitivity: 73%, and specificity: 71%.

cases, levetiracetam in 10 cases, lamotrigine in 9 cases, and other ASDs including phenobarbital, topiramate and etc. in the remaining cases. With regard to NLR1 level, no difference was found between patients who received treatment with ASDs and patients who did not ($P = 0.370$). But, for NLR2 level, it was significantly lower in patients who received treatment with ASDs (Figure 2D, Table 4) than that in patients did not ($P = 0.011$). Finally, NLR1 level was found significantly higher (Figure 2E, Table 4) in patients admitted because of SE than that in patients admitted because of self-limited seizures ($P = 0.013$), but no difference of NLR2 levels was found between these two groups of patients ($P = 0.290$) (Figure 2E, Table 4).

The correlations between NLR level and seizure severity

Seizure severity was assessed by NHS3 scale scores in this study. To evaluate the correlation between NLR level and seizure severity, we found that NLR level was positively correlated with NHS3 scores at admission and during follow-up (Spearman's correlation r = 0.441, $P < 0.001$, and r = 0.221, $P = 0.009$, respectively) (Figures 3A,B).

Discussions

In this retrospective study, 115 patients included were all hospitalized because of acute seizures. Among the 115 patients, 51 patients (44.3%) were admitted because of SE and 56 admitted because of newly diagnosed epilepsy. Forty eight patients were accepting treatment with ASDs before admission. The type of seizures was mainly generalized tonic-clonic seizures.

Studies have indicated an involvement of leukocyte in the pathophysiology of epilepsy (16, 29, 30). The function of peripheral immune cell subsets is altered during epileptic seizures, such as upregulation of CD32-expressed granulocytes and monocytes reduction of HLA-DR-expressed monocytes after seizures (30), and this monocyte infiltration may lead to the recurrence of epileptic seizures (31). On the other hand, a significant increase in the number of blood leukocytes after seizures has been reported in patients with generalized or focal onset seizures, with peripheral blood leukocyte counts above the upper limit of normal in approximately one-third of patients after generalized onset seizures (16, 32). In this retrospective cohort study, we demonstrated that, compared to healthy controls, PWE had higher levels of leukocyte and NLR at admission within 24 h of acute seizure and during the follow-up period. Furthermore, multivariate logistic regression analysis illustrated that the levels of admission leukocyte and NLR1 were both independently associated with acute epileptic seizures. This indicated that inflammation reflected by NLR was involved in the pathophysiology of epilepsy. It is reported that serum albumin can extravasate from blood vessel into the brain parenchyma when the blood-brain barrier is dysfunctional, and this has been suggested to be involved in the pathogenesis of various types of epilepsy (33–35). In this study, we found that low serum albumin was associated with acute seizures, which effectively confirmed albumin extravasation after acute seizures.

Meanwhile, NLR1 level was found to be a significant predictor for recurrent epileptic seizures after admission in this study. According to the ROC analysis for NLR1, we found that the best cut-off value was 5.56 and the AUC was 0.717 (95% CI: 0.611–0.823, $P < 0.001$) with a sensitivity of 73% and a specificity of 71%. This finding suggested that an elevated NLR shortly after an acute seizure may predict a tendency for recurrent seizures in the following days, which indicates that the inflammation reflected by NLR may play a causative role in epileptogenesis. Multiple studies have shown that there is a reciprocal interaction between inflammation and epilepsy (9, 10, 13). Epileptic seizures can disrupt the blood-brain barrier and activate a variety of cells including microglia, astrocytes, mononuclear macrophages and neutrophil in the CNS. Subsequently, the activated microglia, reactive astrocytes and infiltrating immune cells release large amounts of pro-inflammatory mediators including IL-6, IL-1β, TNF-α, COX-2, HMGB1 and chemokines, inducing neuroinflammation through multiple signaling pathways. And the neuroinflammation can further increase excitability of the CNS, along with serum albumin extravasation (14, 35, 36). Thus, neuroinflammation can aggravate the severity, duration, and frequency of epileptic seizures and even lead to new-onset seizures (13, 15, 37). On the other hand, patients with systemic autoimmune diseases, including systemic lupus erythematosus (SLE) and Hashimoto's

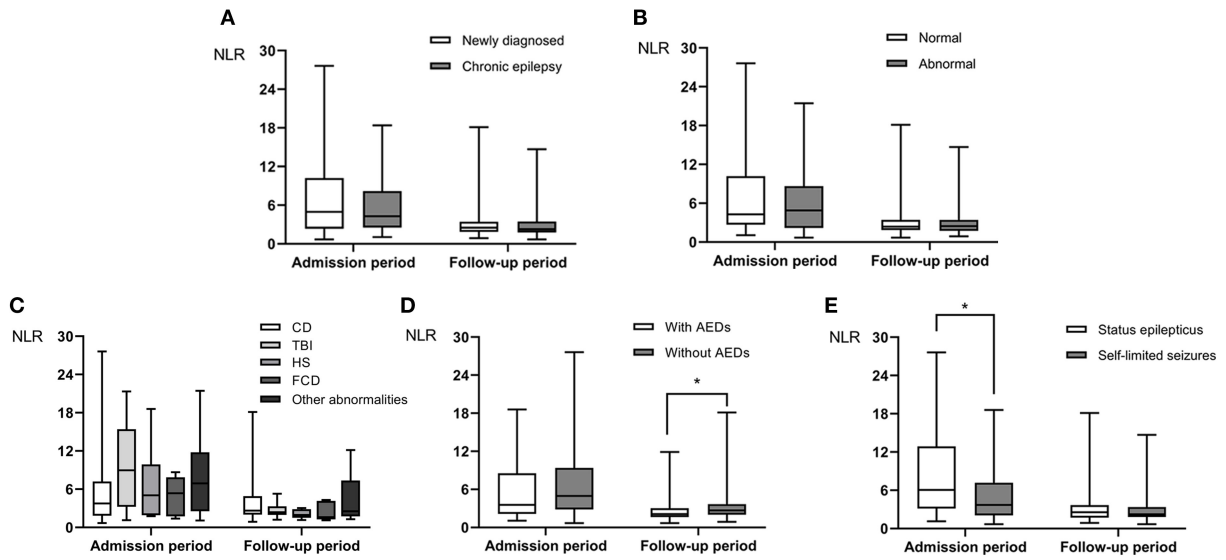

FIGURE 2
The difference in NLR levels at admission (NLR1, within 24 h of acute seizure) and follow-up period (NLR2, 5–14 days after admission) in PWE. Neither NLR1 nor NLR2 level was different between patients with newly diagnosed epilepsy and those with chronic epilepsy **(A)**. There was no significant difference in NLR level between patients with idiopathic epilepsy and those with secondary epilepsy **(B)**, and there was no significant difference in NLR level among the five categories of patients with focal epilepsy **(C)**. NLR2 level in patients on treatment with ASDs was lower than those in patients on no treatment with ASDs **(D)**. NLR1 level in patients with SE was higher than that in patients with self-limited seizures **(E)**. Group comparison was done by Mann-Whitney U test. $*P < 0.05$.

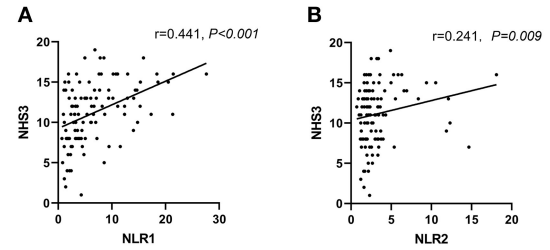

FIGURE 3
Levels of NLR were positively correlated to NHS3 scores in PWE at admission (within 24h of acute seizure) **(A)** and follow-up period (5–14 days after admission) **(B)**. Correlation analysis was done by Spearman's correlation.

thyroiditis, especially children have a significantly increased risk of epilepsy (38). These results suggest that epilepsy is associated with systemic inflammation that can lead to abnormal neuronal connection and neuronal hyperexcitability, thereby mediating the development of epilepsy (10, 17).

In this study, we also found that NLR and leukocyte levels were higher within 24 h of acute seizure than during the follow-up period of 5–14 days after admission. Consistent with previous studies, the increase in NLR level induced by acute seizures subsequently decreased (24), but NLR level remained higher than normal controls during the follow-up period of 5–14 days after admission. From the literature review, most of the previous studies concerning the relationship between epilepsy and NLR were conducted during the acute and subacute stages of seizures, with a maximum follow-up time of 96 h (23, 24). This study is the first to investigate temporal changes in NLR levels during a follow-up period of 5–14 days after an acute epileptic seizure. We found that systemic inflammatory responses reflected by NLR remained increased during 5–14 days of follow-up after acute seizures compared with healthy controls. Some studies have also found that NLR level is higher in patients with chronic temporal lobe epilepsy than in healthy controls (39). Accordingly, it is suggested that the long-lasting systemic inflammatory response induced by acute seizures may be involved in the process of epileptogenesis either in the acute stages or in the chronic stages.

Our study found that there was no difference in NLR levels between the group of patients with newly diagnosed epilepsy and the group of patients with chronic epilepsy, nor between the group of patients with normal brain MRI and the group of patients with focal seizures caused by CD, TBI, HS, FCD and other lesions. This may indicate that the seizure-induced inflammatory response reflected by blood NLR levels is similar in different epileptic causative conditions. Whereas, it should be noted that several studies have shown that the levels of inflammatory cytokines vary with the etiology and historical duration of epilepsy (11, 40). The reason for this inconsistency may be the different inclusion criteria of our study subjects,

or the different of our study to reflect inflammatory response compared with literature reports. Thus, our results suggest that, at least in part, NLR levels depend neither on the simple cumulative effect of recurrent seizures nor on the etiology of epilepsy.

In this study, most patients were already on treatment with ASDs before admission, which mainly included valproic acid, lamotrigine, levetiracetam and carbamazepine. We found that NLR2 level, i.e., NLR level during the follow-up period of 5–14 days after admission, was lower in patients on medications with ASDs than that in patients not on medications with ASDs. On the other hand, previous studies had demonstrated the anti-inflammatory effects of ASDs in epilepsy treatment (41–44). Therefore, we speculate that ASDs may effectively promote the return of NLR to baseline, thereby facilitating the recovery of acute seizure-induced inflammation.

SE is an acute and potentially life-threatening emergency with high morbidity and mortality (45). Proinflammatory events in the periphery or brain have been shown to govern SE occurrence, and SE-induced neurological dysfunction and even death are significantly associated with SE duration (37, 46). In this study, we found that NLR1 level was higher in patients admitted with SE than that in patients admitted with self-limited seizures, implicating that SE induces more severe inflammatory response compared with self-limited seizures. This is consistent with a report that half of the patients developed systemic inflammatory response syndrome (SIRS) after SE, which is considered to be an independent risk factor for drug resistance and death (47). Therefore, routine inflammation assessment, especially NLR assessment, should be performed in SE patients to assess the prognosis of epilepsy and intervene as early as possible.

For patients with uncontrolled epilepsy, seizure severity may be more important than seizure frequency (48). The severity of epilepsy can be assessed by various scales in clinical practice. In this study, NHS3, a valid and easy-to-apply measure, was used to quantitatively assess epilepsy severity (27, 28). We found a significant correlation between NLR levels and NHS3 scores not only within 24 h of acute seizure but also during the follow-up period of 5–14 days after admission. Our study is the first to demonstrate a correlation between NLR level and epilepsy severity, suggesting that NLR is a biomarker for seizure severity. Patients with epilepsy often experience recurrent seizures and generalized tonic-clonic seizures greatly increase the risk of SUDEP depending on the severity of epilepsy (6, 49). Together with the findings that NLR1 level was independently associated with acute seizures and an elevated NLR shortly after an acute seizure may predict a tendency of recurrent seizures in the following days, we believe that monitoring of NLR level can help to assess the severity of acute attacks, so as to identify patients at high risk for developing subsequent seizures and implement effective interventions early to reduce morbidity and mortality.

This study has the following limitations. First, because of its retrospective design, a large number of patients without NLR data were excluded. Second, we recorded NLR2 from a single measure over 5–14 days because the sample size was not large enough. The dynamic changes of neutrophils, lymphocytes and NLR levels can be evaluated more accurately with a larger sample size in the future.

Conclusions

In conclusion, we found that epileptic seizures were associated with inflammatory responses reflected by leukocyte and NLR levels. NLR1 level was independently associated with acute epileptic seizures. Meanwhile, NLR1 level was an important predictor of seizure recurrence after admission. Therefore, NLR-reflected inflammatory responses is a consequence of seizures and may also be responsible for the development of epilepsy. Furthermore, NLR1 and NLR2 levels were respectively associated with SE and use of ASDs, and there was a positive correlation between NLR level and seizure severity, indicating that NLR may be used as a biomarker for seizure severity. We suggest that monitoring of NLR levels should be performed as early as possible to help predict the risk of seizure recurrence following acute epileptic seizure attack and intervene as early as possible.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding authors.

Ethics statement

The studies involving human participants were reviewed and approved by Ethics Committee of The First Affiliated Hospital of Anhui Medical University. Written informed consent from the participants' legal guardian/next of kin was not required to participate in this study in accordance with the National Legislation and the Institutional Requirements.

Author contributions

HL explained the data and wrote the paper. YY, MH, and XC acquired and analyzed data. CD, QS, and RLi performed the literature search and data collection. RLiu and XX contributed data curation and investigation. HL and YW designed the

study and revised the manuscript. All authors approved the final manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Relationship between brain activity, cognitive function, and sleep spiking activation in new-onset self-limited epilepsy with centrotemporal spikes

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Objective: This study aimed to investigate the relationship between cognitive function sleep spiking activation and brain activity in self-limited epilepsy with centrotemporal spikes (SeLECTS).

Methods: We used spike-wave index (SWI), which means the percentage of the spike and slow wave duration to the total non-REM (NREM) sleep time, as the grouping standard. A total of 14 children with SeLECTS (SWI ≥ 50%), 21 children with SeLECTS (SWI < 50%), and 20 healthy control children were recruited for this study. Cognitive function was evaluated using the Wechsler Intelligence Scale for Children, Fourth Edition (Chinese version) (WISC-IV). Magnetic source activity was assessed using magnetoencephalography calculated for each frequency band using the accumulated source imaging (ASI) technique.

Results: Children with SeLECTS (SWI ≥ 50%) had the lowest cognitive function scores, followed by those with SeLECTS (SWI < 50%) and then healthy controls. There were significant differences in the localization of magnetic source activity between the three groups: in the alpha (8–12 Hz) frequency band, children with SeLECTS (SWI ≥ 50%) showed deactivation of the medial frontal cortex (MFC) region; in the beta (12–30 Hz) frequency band, children with SeLECTS (SWI ≥ 50%) showed deactivation of the posterior cingulate cortex (PCC) segment; and in the gamma (30–80 Hz) frequency band, children in the healthy group showed activation of the PCC region.

Conclusion: This study revealed significant decreases in cognitive function in children with SeLECTS (SWI ≥ 50%) compared to children with SeLECTS (SWI < 50%) and healthy children, as well as significant differences in magnetic source activity between the three groups. The findings suggest that deactivation of magnetic source activity in the PCC and MFC regions is the main cause of cognitive function decline in SeLECTS patients with some frequency dependence.

KEYWORDS

electrical status epilepticus of sleep (ESES), magnetoencephalography (MEG), cognitive function, brain activity, self-limited epilepsy with centrotemporal spikes, sleep spiking activation

Introduction

Self-limited epilepsy with centrotemporal spikes (SeLECTS), which is also known as Rolandic epilepsy (RE) and Benign childhood epilepsy with centrotemporal spikes (BECTS), is the most common type of childhood idiopathic epilepsy (1). The International League Against Epilepsy (ILAE) has changed the term "benign" to "self-limited" in the latest version of the definition in 2022, taking into account that the typical evolution of this type of epilepsy is age-related onset and remission (2, 3). In China, the mean age of onset of SeLECTS is 6.85 years and the male to female ratio is 6:4 (4). Typical clinical presentation is limited motor-sensory seizures on one side of the mouth and face, with extension to generalized tonic-clonic seizures (5). In these patients, electroencephalogram (EEG) commonly shows normal background activity and spike discharges in the centrotemporal region with high amplitude that are usually more pronounced during sleep (6). While SeLECTS was previously thought to have a favorable prognosis, a growing number of studies suggest that, even in adulthood when patients are seizure-free, SeLECTS is associated with greater neuropsychiatric dysfunction (7–9).

Children with SeLECTS show significantly more discharges during sleep, some of which reach electrical status epilepticus (ESES) (10). This group of patients is characterized by a poor prognosis and a negative impact on the development of cognitive function (11). It is widely believed that frequent epileptiform discharges affect the formation and establishment of synapses, damage neural circuits, and produce changes in the structure and function of brain tissue that affect the entire functional network of the brain and lead to cognitive decline (12–14). In a clinical study, Overvliet et al. (15) found an association between cognitive impairment and nocturnal epileptiform discharges. This association was interpreted as a disruption of the functional connections responsible for the corresponding cognitive ability. In a functional magnetic resonance imaging (fMRI) study of nine children with SeLECTS with ESES, we observed alterations in the salience network (SE) and central executive network (CEN) (10).

ESES is an interictal EEG pattern that occurs during slow wave sleep (16). ESES patterns usually consist of continuous, symmetrical spike patterns with variable discharge frequencies, usually in the range of 1.5–3 HZ. The spike-wave index (SWI), which is the percentage of the spike and slow wave duration to the total non-REM (NREM) sleep time, is commonly used to measure the severity of ESES (17). In general, the higher the SWI, the more epileptiform discharges and the more severe the cognitive impairment (18). It is now generally accepted that a SWI of ≥85% can be considered ESES, but some studies have also shown that cognitive and behavioral impairment can occur even with a low percentage of sleep. So some authors also use lower SWI values as a cut-off (19, 20), or consider that a SWI of at least ≥50 should trigger the possibility of an ESES related syndrome (21). In the current study, we decided to divide patients in two groups according to a SWI threshold at 50%, and all EEG interpretations were performed by two experienced EEG physicians.

Magnetoencephalography (MEG) is a non-invasive neuroimaging method with ultra-high spatial and temporal resolution (22). Compared to EEG, the magnetic field is not attenuated as it passes through the skull and scalp, allowing for clearer detection of brain magnetic activity (23). MEG can capture higher frequency information compared to fMRI, which can only retain low frequency information (24, 25). Given the frequency-dependent nature of brain activity (26), MEG can be used to more accurately localize neuromagnetic brain activity in each frequency band.

Previous studies have investigated differences in cognitive function in children with SeLECTS in longitudinal comparisons, as well as differences before and after medication administration (27–29). However, few studies have compared the cognitive function of children with SeLECTS without antiepileptic drugs (AEDs) in a cross-sectional manner with different evolutionary patterns, especially using the MEG technique (30, 31). We use EEG as a basis for grouping because children with developmental epileptic encephalopathy with spike-and-wave activation in sleep (DEE-SWAS) and epileptic encephalopathy with spike-and-wave activation in sleep (EE-SWAS), as defined by the latest ILAE definitions, whose diagnostic criteria include significant behavioral regression and negative myoclonus in addition to EEG manifestations, are difficult to diagnose at first presentation. Most of the patients diagnosed were already taking AEDs, which interfered with the results of this experiment. Therefore, in this trial we used only the EEG, an objective indicator, as a basis for grouping. In the present study, we compared the intensity and localization of magnetic sources in different frequency bands in children with SeLECTS (SWI ≥ 50%), children with SeLECTS (SWI < 50%), and healthy control (HC) children. Cognitive function in the three groups was assessed using the Wechsler Intelligence Scale for Children, Fourth Edition (Chinese version) (WISC-IV). We tentatively hypothesized that SeLECTS children (SWI ≥ 50%) would have the worst cognitive function and that there would be differences in magnetic source activity intensity and magnetic source localization between the three groups.

Experimental procedures

Participants

Between October 2020 and October 2021, 21 children were diagnosed with SeLECTS (SWI ≥ 50%) and 24 children were diagnosed with SeLECTS (SWI < 50%) only at the Neurology Department of the Nanjing Children's Hospital and Nanjing Brain Hospital. Of the SeLECTS (SWI < 50%) patients, two were

taking AEDs and one had a history of encephalitis; thus, 21 children were included in this study. Of the SeLECTS patients (SWI $\geq$ 50%), six had a history of AEDs and one had an autism spectrum disorder; thus, only 14 patients were recruited for this study. We also recruited 20 HC children matched with the patients in terms of age, gender, parents educational background, and family socioeconomic status. The inclusion criteria for patients were as follows: (1) a diagnosis of SeLECTS according to the ILAE 2022 classification of epilepsy syndrome (32); (2) no use of AEDs at the time of enrollment; (3) normal brain MRI image; and (4) no other types of epilepsy or other major neuropsychiatric disorders. The exclusion criteria were: (1) did not cooperate when MEG and MRI data were collected which interfered with data collection; (2) brain MRI showed abnormalities; (3) a diagnosis of other types of epilepsy or major neuropsychiatric disorders; and (4) the patients parents did not cooperate with the examination or provide informed consent. All patients were seizure-free for at least 72 h before and 24 h after the MEG and MRI scans were performed.

Based on previous studies (19–21), we hypothesize that there is a significant difference between the cognitive function, brain activity of children with SeLECTS using SWI = 50% as the cut-off. In the SeLECTS (SWI $\geq$ 50%) group, two EEG results for patients within 1 month showing SWI $\geq$ 50%; the SWI (%) was obtained as the total number of minutes of all spike and slow-wave abnormalities divided by the total number of minutes of NREM and multiplied by 100 (33). All patients were subjected to sleep deprivation to better record the EEG during sleep. The recording time was 2 h and the SWI was calculated on 45 min of NREM sleep. EEG reports for all patients issued by two experienced EEG physicians.

This study was approved by the Medical Ethics Committee of Nanjing Medical University, Nanjing Brain Hospital and Nanjing Children's Hospital. In addition, the parents of all participants in the study provided informed consent.

Neuropsychological assessment

For all participants, cognitive function was assessed using the Wechsler Children Intelligence Scale, Fourth Edition (Chinese version) (WISC-IV). The test comprises 14 sub-tests that provide a full-scale intelligence quotient (FSIQ) to account for overall cognitive abilities as well as four additional composite scores that account for cognitive abilities in different areas (34). The four composite scores are the Verbal Comprehension Index (VCI), Perceptual Reasoning Index (PRI), Working Memory Index (WMI), and Processing Speed Index (PSI). The VCI, which includes tests of analogy, vocabulary, comprehension, and general knowledge, measures language learning ability, concept formation, abstract thinking, and analytical generalization. The PRI includes block design, picture concepts, matrix reasoning, and fill-in-the-blank tests, which measure reasoning ability, spatial perception, and visual organization, respectively. The WMI includes a recited number test, alphabetic-numeric alignment, and arithmetic test to measure memory ability, comprehension, and application of external information. The PSI includes decoding tests, symbol retrieval, and scratch tests to measure the speed of understanding simple information and the speed and accuracy of recording (35, 36).

MEG recordings

MEG data were collected in the magnetic shielding room of the MEG Center of Nanjing Brain Hospital using a full-head CTF-275 channel MEG system (VSM Medical Technology Company, Canada). In preparation for data collection, subjects were asked to make sure they were free of any metal objects and to fix three coils at the base of their nose and in front of each ear to determine the position of their head in the MEG system. During data collection, subjects were asked to remain relaxed, stay still, keep their eyes closed, stay awake, and keep their mouth slightly open. If a subject's head moved more than 5 mm during the data collection process, the data were discarded and re-collected. The sampling frequency of MEG was 6,000 Hz and at least six 120 s sessions of MEG data were collected per subject. Noise was eliminated using third-order gradients before data acquisition.

MRI scan

All participants underwent a 3.0 T MRI (Siemens, Germany) scan after MEG data collection. Before MRI scanning, coils similar to those used in MEG data collection were placed at the nose root and in front of both ears to locate each subjects head position.

Data preprocessing

MEG data were processed through the following steps: First, we excluded MEG waveforms with significant noise and artifacts (amplitude $>$ 6 pT), based on previous studies (37). Next, we filtered the MEG data in the frequency range 1–70 Hz and used it to identify high amplitude peaks in SeLECTS. To reduce the impact of spikes, we selected MEG data without spikes for a continuous period of 60 s for processing. Finally, we analyzed the six frequency bands of the selected waveform, namely delta (1–4 Hz), theta (4–8 Hz), alpha (8–12 Hz), beta (12–30 Hz), gamma (30–80 Hz), and ripple (80–250 Hz). The data were filtered before analysis to avoid environmental alternating current (AC) power interference around the 50 Hz band. MEG data from all subjects were analyzed using the MEG processor (https://sites.google.com/site/braincloudx/).

Source localization

Based on previous studies (38–40), we used accumulated source imaging (ASI) to analyze neuromagnetic source activity in multiple frequency bands in the selected MEG data. The sum of the volumes of source activity over a period of time is defined as ASI, which can locate the neuromagnetic sources. ASI can be expressed by the following equation:

$$\mathrm{Asi}(\mathrm{r}, \mathrm{s}) = \sum_{t=1}^{t=n} Q(r, t)$$

Where, ASI represents the accumulated source strength at position r, s indicates the time slice, n represents the time point of the MEG data, and Q represents the source activity at source r and at time point t. We defined $s \geq 1$ and $s \leq n/2$.

We used two-step beamforming to calculate the source activity and locate the source (37). First, we calculated the lead domain for each source (or voxel), which is called a voxel-based partial sensor, and generated the MEG data matrix. Next, to minimize the influence of coherent sources in the localization of the source, we performed a partial sensor overlay for each voxel (37). We then calculated the covariance of all sensors. We calculated two sets of source images using the vector beamformer and estimated the coherent source and source direction using the variance matrix vector beamformer (41). After these steps, we used the scalar beamformer to generate the source activity (or virtual sensor waveform). The algorithm has been described in detail and validated in prior studies (41).

For each subject, the entire brain was scanned at a resolution of 6 mm. In cases where the distance between two voxels was < 10 mm, they were considered as one source. The individual MRI data were merged with the MEG data by fixing the nasal root and three points in front of the ear at the time of data acquisition. This approach allows us to segment and visualize brain regions.

Statistical analysis

Fisher's exact probability method was used to examine the localization of the main neuromagnetic sources in the three groups. One-way analysis of variance (ANOVA) was performed on the source strength, Wechsler scale scores, and subject age. We also conducted a homogeneity test of variance. We compared the age at onset and duration of disease between the SeLECTS (SWI ≥ 50%) group and SeLECTS (SWI < 50%) group using the two-sample *t*-test. Pearson or Spearman correlation analysis was used to analyze the relationship between the intensity of the magnetic source and the clinical characteristics of each group of frequency bands. The level of statistical significance was set at $p < 0.05$ with Bonferroni multiple comparison correction (e.g., for six bands in three groups, $p < 0.002$). All statistical analyses were performed using SPSS version 24.0 for Windows (SPSS Inc., Chicago, IL, USA).

Results

Participants

The final sample included 14 children with SeLECTS (SWI ≥ 50%) including eight females, with a mean age of 96.94 ± 13.68 months, a mean age of onset of 90.89 ± 15.08 months, a mean disease duration of 6.05 ± 6.06 months, and an average of 2.64 ± 1.45 seizures. The 21 children with SeLECTS (SWI < 50%), which included 10 males, had a mean age of 102.59 ± 19.20 months, a mean age of onset of 97.84 ± 16.25 months, a mean disease duration of 5.22 ± 6.68 months, and an average of 2.00 ± 1.18 seizures. The statistical analysis showed no significant differences between the two groups in terms of gender, age, age of onset, disease duration, or number of seizures. The detailed data of the participants are shown in Table 1.

WISC-IV scores

As shown in Table 2, there were statistically significant differences between the three groups in terms of total score and PRI, with the SeLECTS (SWI ≥ 50%) group having the lowest score followed by the SeLECTS (SWI < 50%) group; the HC group had the highest score. In terms of VCI and WMI, both the SeLECTS (SWI ≥ 50%) group and SeLECTS (SWI < 50%) group had significantly lower scores than the HC group, but there was no statistically significant difference between the scores of the SeLECTS (SWI ≥ 50%) group and SeLECTS (SWI < 50%) group. There were no significant group differences between the PSI scores. Additional details of the performance for each group are provided in Figure 1.

Source location

According to the whole brain accumulative magnetic source imaging, there were 1–3 primary magnetic source locations per subject in the resting state (Figure 2), including the peri-Rolandic area (PR), posterior cingulate cortex (PCC), medial frontal cortex (MFC), medial temporal lobe (MTL), and deep brain area (DBA). In the low frequency band (<80 Hz), there was a significant difference in source localization between the three groups; however, in the high frequency band (>80 Hz), there was not. Specifically, in the alpha (8–12 Hz) band, the SeLECTS (SWI < 50%) group and HC group were mainly localized in the MFC and PCC regions, while the magnetic source in the SeLECTS (SWI ≥ 50%) group was mainly localized in the PCC region. In the gamma band (30–80 Hz), the SeLECTS

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TABLE 1 Demographic and clinical characteristics of the included participants.

Participant	Gender	Age (months)	Epilepsy duration (months)	Age at onset (months)	Number of seizures	SWI
1[a]	F	106	5.8	100.2	4	50–60%
2[a]	M	86.1	5.8	80.3	2	55%
3[a]	F	105.5	2	103.5	2	65%
4[a]	M	103.2	4.4	98.8	3	60%
5[a]	F	97.2	24	73.2	5	65–70%
6[a]	M	72.6	8.3	64.3	1	70%
7[a]	F	82.4	2.5	79.9	6	70%
8[a]	F	112.5	12.5	100	2	50%
9[a]	F	116.3	2.6	113.7	2	50%
10[a]	M	95.9	3	92.9	3	60%
11[a]	F	117	2.5	114.5	2	65%
12[a]	F	91.8	2.2	89.6	1	50–60%
13[a]	M	85.4	8	77.4	2	60%
14[a]	M	85.2	1.1	84.1	2	50%
15	F	84.8	12	82.8	2	40%
16	M	120.6	12	108.6	3	30%
17	F	132.8	24	108.8	2	30%
18	M	114.5	5.4	109.1	5	35%
19	F	73.5	4	69.5	4	30%
20	M	101.5	1	100.5	2	30%
21	F	99.5	4	95.5	2	25%
22	M	107.3	9.3	98	1	40%
23	M	85	1.8	83.2	1	20%
24	F	99.4	0.6	98.8	1	20%
25	F	74.6	0.7	73.9	1	40%
26	M	131.8	0.6	131.2	3	30%
27	F	96.9	0.6	96.3	1	10%
28	M	135.8	18.4	117.4	1	10%
29	M	110.8	10	100.8	2	15%
30	M	76.8	2.8	74	4	25%
31	F	113.3	0.4	112.9	2	40%
32	M	77.5	0.3	77.2	2	40%
33	F	99.4	0.6	98.8	1	20%
34	F	101.1	0.3	100.8	1	40%
35	F	117.4	0.9	116.5	1	35%

SWI, spike-wave index; [a]SeLECTS (SWI $\geq$ 50%) group.

(SWI $\geq$ 50%) group and SeLECTS (SWI < 50%) group were mainly localized in the MFC region, while the HC group was mainly localized in the MFC and PCC regions. In the beta (12–30 Hz) frequency band, we found group differences in localization in the PCC region. *Post-hoc* analysis showed a statistically significant difference in localization in the SeLECTS (SWI $\geq$ 50%) group compared to the SeLECTS (SWI < 50%) group and HC group, but no statistically significant difference between the SeLECTS group and healthy controls.

We did not find significant differences in the other three frequency bands. However, as shown in Figure 2, in the higher frequency bands the magnetic source activity is more frequent in the DBA region than in the lower frequency bands, while in the lower frequency bands the magnetic source activity in the PR region is more frequent than in the higher frequency bands. The localization and statistical comparison of the main magnetic sources in each frequency band for the three groups are detailed in Table 3.

TABLE 2 Comparison of WISC-IV scores between the SeLECTS (SWI ≥ 50%), SeLECTS (SWI < 50%), and HC groups.

WISC-IV	SeLECTS (SWI ≥ 50%) (Group 1, $n = 14$)	SeLECTS (SWI < 50%) (Group 2, $n = 21$)	HC (Group 3, $n = 20$)	p	P: Group 1 vs. Group 2	P: Group 1 vs. Group 3	P: Group 2 vs. Group 3
FSIQ	92.21 ± 6.879	103.94 ± 5.117	119.10 ± 10.586	0.00*	0.000**	0.000**	0.000**
VCI	84.64 ± 8.705	96.94 ± 14.263	114.1 ± 15.864	0.00*	0.019	0.000**	0.004**
PRI	102.57 ± 9.387	111.65 ± 6.214	120.7 ± 10.489	0.00*	0.007**	0.000**	0.004**
WMI	90.64 ± 7.652	99.29 ± 8.252	113.3 ± 18.576	0.00*	0.076	0.000**	0.002**
PSI	99.21 ± 14.818	104.71 ± 8.73	107.15 ± 9.549	0.125	0.553	0.253	0.807

FSIQ, Full-scale Intelligence Quotient; VCI, verbal comprehension index; PRI, perceptual reasoning index; WMI, working memory index; PSI, processing speed index. *$p < 0.05$ after one-way analysis of variance (ANOVA); **$p < 0.0167$ after Bonferroni correction for multiple comparisons.

Source strength

In the delta (1–4 Hz) frequency band, the magnetic source intensity of the SeLECTS (SWI ≥ 50%) group was significantly higher than that of the SeLECTS (SWI < 50%) group and HC group. However, there was no significant difference between the magnetic source intensity of the SeLECTS (SWI < 50%) group and HC group. In the alpha (8–12 Hz) and gamma (30–80 Hz) frequency bands, we found a statistically significant difference in the magnetic source intensity between the three groups; however, the pairwise comparison showed no statistically significant difference between the two groups. In the other three frequency bands, there were no statistically significant group differences. Detailed statistical analysis of the neuromagnetic sources is shown in Table 4.

Clinical correlation

Our analysis revealed no significant correlation between the magnetic source intensity and clinical data in the SeLECTS (SWI ≥ 50%) group and SeLECTS (SWI < 50%) group. Similarly, there was no significant correlation between the age of onset and disease duration in the two groups.

Discussion

This study has revealed significant differences in neuropsychological evaluation results between unmedicated children with SeLECTS (SWI < 50%) and unmedicated children with SeLECTS (SWI ≥ 50%). Similarly, there were significant differences in magnetic source localization and magnetic source intensity between these two groups compared to the HC group, and they showed some frequency dependence. This is tentatively consistent with our hypothesis that frequent discharges during sleep would alter normal brain magnetic source activity and thus affect cognitive function. To the best of our knowledge, this is the first MEG study of unmedicated SeLECTS (SWI < 50%), unmedicated SeLECTS (SWI ≥ 50%), and HC subjects, as previous studies have mainly used fMRI and focused on relevant functional networks of the brain (42–44).

Clinical data and characteristics

Previous studies (10, 44) suggest that children with SeLECTS (SWI ≥ 50%) have an earlier age of onset, longer disease duration, and more seizures than children with SeLECTS (SWI < 50%). However, we found no such patterns in our sample. During patient recruitment, we found that parents who become more aware of epilepsy disorders are inclined to go to the hospital immediately for even minor petit mal seizures or nocturnal episodes. This means that, regardless of the outcome of SeLECTS, in most cases patients were recruited at the time of the first one or two seizures, resulting in no statistically significant difference between the two groups in terms of duration of illness and number of seizures. However, at follow-up, we found that children with SeLECTS (SWI ≥ 50%) were not as well controlled as children with SeLECTS (SWI < 50%), even with use of AEDs, and continued to have seizures. This further confirms that children with SeLECTS (SWI ≥ 50%) have poorer outcomes, more difficult seizure control, and lower neuropsychological scores (42).

We found no significant difference in the age of onset of SeLECTS (SWI ≥ 50%) compared to SeLECTS (SWI < 50%) children, which is likely because of the sample size. In addition, we did not find a significant correlation between the age at onset and duration of illness. We do not believe that there is a simple linear relationship between magnetic source intensity, age at onset, number of episodes, and duration of illness, which we plan to reconfirm in a larger sample (45).

Neuropsychological test results

We found that SeLECTS (SWI < 50%) patients and SeLECTS (SWI ≥ 50%) scored significantly lower than healthy controls on both the VCI and WMI, although there was no significant difference between the two patient groups. This finding is similar

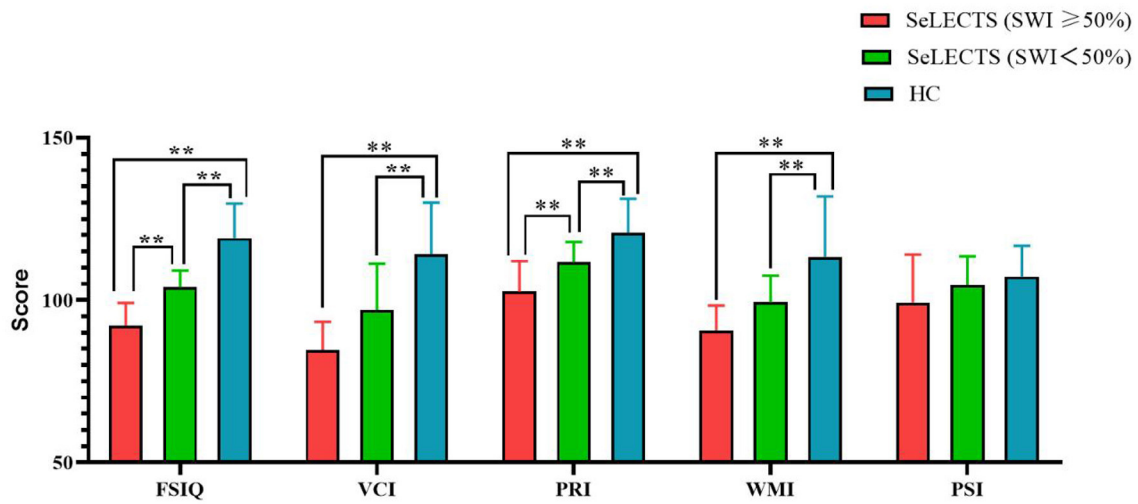

FIGURE 1
Comparison of WISC-IV scores between the SeLECTS (SWI ≥ 50%), SeLECTS (SWI < 50%), and HC groups. FSIQ, Full-scale Intelligence Quotient; VCI, verbal comprehension index; PRI, perceptual reasoning index; WMI, working memory index; PSI, processing speed index. **, The *p*-value was statistically significant.

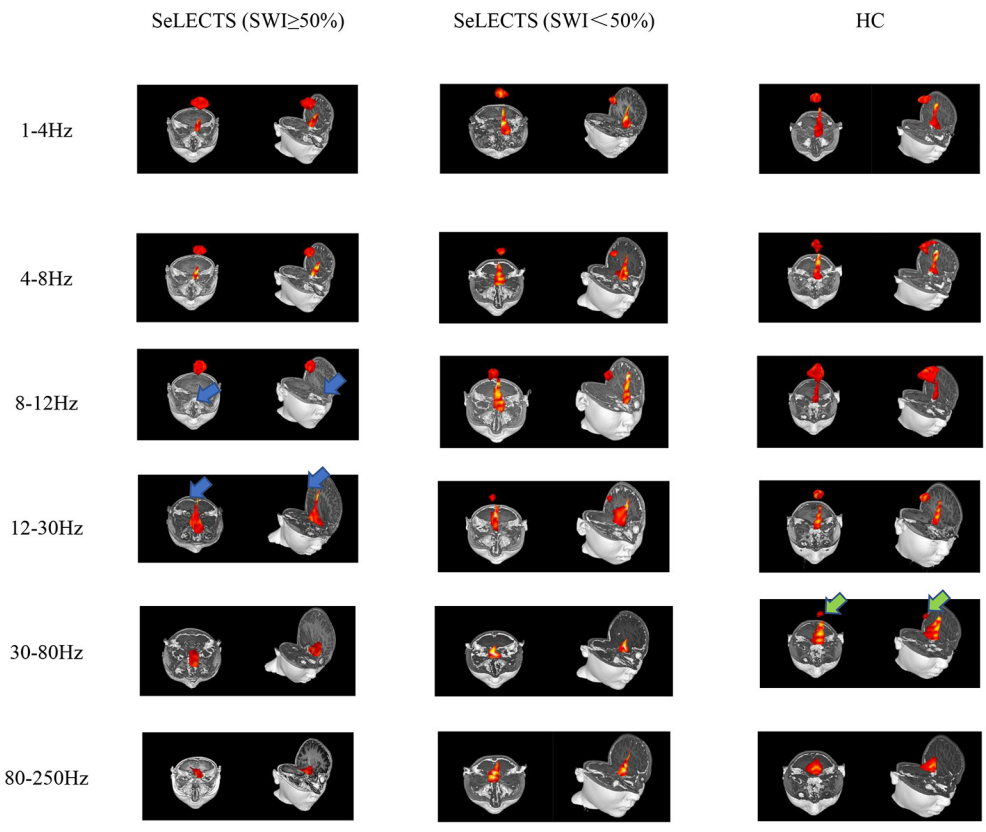

FIGURE 2
Typical magnetic source localization in the frequency band of 1–250 Hz for three groups of subjects. In the 8–12 and 12–30 Hz frequency bands, the SeLECTS (SWI ≥ 50%) group was inactivated in the PCC region, as indicated by the blue arrows. In the 30–80 Hz band, the HC group is activated in the PCC region as shown by the green arrow.

TABLE 3 Comparison of predominant neuromagnetic activity in the SeLECTS (SWI ≥ 50%), SeLECTS (SWI < 50%), and HC groups.

Frequency band (Hz)	Source location	SeLECTS (SWI ≥ 50%) (Group 1, $n = 14$)	SeLECTS (SWI < 50%) (Group 2, $n = 21$)	HC (Group 3, $n = 20$)	p	P: Group 1 vs. Group 2	P: Group 1 vs. Group 3	P: Group 2 vs. Group 3
1–4 Hz	PR	3	5	2	0.556			
	PCC	5	5	10	0.222			
	MFC	6	16	13	0.143			
	MTL	2	1	2	0.623			
	DBA	1	2	2	1.000			
4–8 Hz	PR	3	4	2	0.723			
	PCC	4	11	9	0.404			
	MFC	6	13	13	0.451			
	MTL	3	1	0	0.078			
	DBA	2	2	1	0.839			
8–12 Hz	PR	2	3	4	0.902			
	PCC	9	14	12	0.937			
	MFC	3	15	15	0.003*	0.006**	0.004**	1.000
	MTL	2	0	0	0.061			
	DBA	2	2	1	0.839			
12–30 Hz	PR	4	3	3	0.608			
	PCC	1	11	11	0.009*	0.01**	0.009**	1.000
	MFC	6	16	14	0.118			
	MTL	2	2	0	0.287			
	DBA	1	4	2	0.675			
30–80 Hz	PR	0	0	0	-			
	PCC	1	1	10	0.001*	1.000	0.011**	0.001**
	MFC	11	19	18	0.596			
	MTL	1	3	0	0.231			
	DBA	2	6	3	0.522			
80–250 Hz	PR	0	0	0	-			
	PCC	0	0	0	-			
	MFC	10	11	11	0.541			
	MTL	3	8	6	0.617			
	DBA	4	7	5	0.929			

PR, peri-Rolandic area; PCC, posterior cingulate cortex; MFC, medial frontal cortex; MTL, medial temporal lobe; DBA, deep brain area.* $p < 0.05$ after Fishers exact test; ** $p < 0.0167$ after Bonferroni correction for multiple comparisons. The number in the columns represents the number of patients showing this source localization.

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TABLE 4 Comparison of neuromagnetic source strength between the SeLECTS (SWI ≥ 50%), SeLECTS (SWI < 50%), and HC groups.

Frequency band (Hz)	SeLECTS (SWI ≥ 50%) (Group 1, $n = 14$)	SeLECTS (SWI < 50%) (Group 2, $n = 21$)	HC (Group 3, $n = 20$)	p	P: Group 1 vs. Group 2	P: Group 1 vs. Group 3	P: Group 2 vs. Group 3
1–4 Hz	89.97 ± 9.13	78.52 ± 5.64	76.34 ± 4.29	0.00*	0.001**	0.000**	0.432
4–8 Hz	79.32 ± 6.18	83.8 ± 5.3	81.55 ± 5.48	0.385	0.461	0.641	0.913
8–12 Hz	86.31 ± 12.38	79.10 ± 6.87	89.91 ± 5.61	0.05*	0.355	0.191	0.015
12–30 Hz	72.64 ± 5.28	72.82 ± 7.52	70.51 ± 4.28	0.414	0.931	0.311	0.221
30–80 Hz	53.86 ± 3.7	51.72 ± 4.54	56.51 ± 7.21	0.03*	0.357	0.429	0.048
80–250 Hz	39.21 ± 2.04	39.79 ± 3.2	41.29 ± 4.35	0.187	0.888	0.201	0.521

*$p < 0.05$ after one-way analysis of variance (ANOVA); **$p < 0.002$ after Bonferroni correction for multiple comparisons.

to the results of previous scales assessing SeLECTS patients with unilateral and bilateral discharges (46). In the SeLECTS (SWI ≥ 50%) group, four patients had bilateral discharges; in the SeLECTS (SWI < 50%) group, all 21 had unilateral discharges. However, we did not find a correlation between SeLECTS (SWI ≥ 50%) and bilateral discharges. We hope to expand the sample size in a future study. In terms of total score and PRI, not only did the SeLECTS (SWI ≥ 50%) and SeLECTS (SWI < 50%) group score significantly lower than the HC group, the SeLECTS (SWI ≥ 50%) group also scored significantly lower than the SeLECTS (SWI < 50%) group. There is growing evidence (44, 47, 48) that, because the brain is at a critical stage of development during childhood, frequent epileptiform discharges during this period can affect the formation and development of neural protrusions, leading to widespread cognitive impairment in children with SeLECTS. The neural network theory of epilepsy suggests that abnormal discharges during the interictal period may affect the neuropsychological development of children more than the clinical seizures themselves (9, 49, 50). This could explain why children with SeLECTS (SWI ≥ 50%) have worse cognitive function than SeLECTS (SWI < 50%) patients and healthy controls (42, 44); the higher the number of discharges during the interictal period, the greater the impact on cognitive function.

Our findings showed no significant difference in PSI between the SeLECTS (SWI ≥ 50%) group, SeLECTS (SWI < 50%) group, and healthy controls, which is inconsistent with previous studies of cognitive function in patients with initially diagnosed SeLECTS (35). However, some studies have reported non-significant differences between SeLECTS (SWI < 50%) patients and healthy controls in terms of PSI (46). A follow-up study of SeLECTS patients taking medication for 1 year found no significant increase in PSI over the1 year period (51). In terms of overall trends, we found that the PSI scores and mean scores of children with SeLECTS (SWI ≥ 50%) and SeLECTS (SWI < 50%) were lower than those of healthy controls, but it did not reach the level of statistical significance. Thus, we plan to expand the sample size for further research.

Source localization

The magnetic source analysis revealed that the SeLECTS (SWI ≥ 50%) group showed extensive deactivation of the PCC and MFC regions, specifically in the MFC region in the alpha (8–12 Hz) frequency band and in the PCC region in the beta (12–30 Hz) frequency band. In addition, in the gamma (30–80 Hz) frequency band, the SeLECTS (SWI ≥ 50%) group and SeLECTS (SWI < 50%) group showed inactivation in the PCC region. Although a clear mechanism of cognitive decline in children with SeLECTS has not been identified, some studies suggest that frequent interictal discharges can lead to altered magnetic sources in important brain regions, thus affecting the corresponding functions (40, 52, 53).

The brain functions with network properties that dynamically regulate information interactions between various systems. The default mode network (DMN) is an important network that maintains the basic state of the nervous system, remaining active during resting states and being inhibited during working states or in the presence of external stimuli. The DMN consists mainly of discrete, bilateral, and symmetrical cortical areas located in the medial and lateral parietal, medial prefrontal, and medial and lateral temporal cortices of the brain (54, 55). The MFC and PCC are well-known as core regions of the DMN, and the SeLECTS (SWI ≥ 50%) and SeLECTS (SWI < 50%) groups showed deactivation of MFC and PCC regions in the corresponding frequency bands, suggesting that DMN impairment is an important cause of cognitive decline in children with SeLECTS (56, 57).

Previous studies have found that prefrontal regions in patients with SeLECTS are associated with neuropsychological deficits (10). A study of brain white matter in patients with SeLECTS similarly found that reduced function of frontal regions may be responsible for cognitive impairment (58). In the present study, the SeLECTS (SWI ≥ 50%) group under the alpha frequency band showed deactivation in the MFC region compared to the SeLECTS (SWI < 50%) and HC groups, which explains why the SeLECTS (SWI ≥ 50%) group had poorer cognitive function. Similarly, a study by Li et al. (45) found that

magnetic source activity in children with SeLECTS with poorer cognitive function was deactivated in the MFC region.

In a study of changes in brain magnetic activity and cognitive function in SeLECTS patients before and after AEDs, it was found that children with SeLECTS showed activation of the PCC region in several frequency bands after treatment, especially the gamma frequency band. Treated SeLECTS patients also showed significant improvement in VCI and PRI scores, suggesting that PCC regions are closely related to verbal comprehension and perceptual reasoning functions (51). Similarly, our study found that both the SeLECTS (SWI $\geq$ 50%) and SeLECTS (SWI < 50%) groups were inactivated in the PCC region in the gamma frequency band compared to the HC group. In the beta frequency band, only the SeLECTS (SWI $\geq$ 50%) group showed deactivation in the PCC region, while the SeLECTS (SWI < 50%) and HC groups showed activation, suggesting that PCC deactivation in multiple frequency bands affects, not only a specific band, affects patients cognitive function.

Previous studies (55, 56) suggest that network reorganization of the DMN leads to altered cognitive function in SeLECTS patients. Thus, in the present study, we hypothesized a causal relationship between magnet source inactivation and network reorganization, and that magnet source inactivation would lead to impaired corresponding functional connectivity of the DMN (45). In addition, we found no significant difference in the number of seizures between the SeLECTS (SWI $\geq$ 50%) and SeLECTS (SWI < 50%) groups. However, there was a significant difference in cognitive function between the two groups, which suggests that frequent nighttime sleep discharges are the main cause of cognitive decline in SeLECTS patients, rather than seizures.

Notably, not only in the SeLECTS group, but also in a MEG study of Childhood absence epilepsy (CAE), we found that the higher the frequency band, the more the magnetic source activity in epileptic patients tended to be distributed in the deep brain; the exact mechanism of this has not yet been clarified (40). In the future, we plan to analyze magnetic source activity in higher frequency bands by expanding the sample size and using more sophisticated MEG processing software.

Source strength

In the delta (1–4 Hz) frequency band, the magnetic source intensity in the SeLECTS (SWI $\geq$ 50%) group was significantly higher than that in the SeLECTS (SWI < 50%) group and HC group. In a prior MEG study (35) examining the cognitive function of children with SeLECTS not taking AEDs, we found that, in the gamma (30–80 Hz) frequency band, the magnetic source activity intensity was highest in SeLECTS children in the group with poorer cognitive function. These results are consistent with a previous MEG study (41) showing that increased magnetic source intensity in children with epilepsy during the interictal period was attributed to frequent discharges. Another MEG study (53) of interictal discharges in SeLECTS confirmed our finding that patients with epilepsy who had interictal discharges had higher magnetic source intensity in the delta (1–4 Hz) frequency band.

Limitations

This study is subject to several limitations. Firstly, the sample size for each group was relatively small. We plan to expand the sample size to validate the experimental results in future studies. Secondly, because unable to assess the patient's eventual cognitive decline, we were making impossible to make a diagnosis of DEE-SWAS and EE-SWAS, and we will more fully document the patient's seizures and subsequent cognitive decline in a follow-up study. As a third point, we artifacts in EMG, ECG, and other signals may interfere with the acquisition of MEG information, although we utilized various methods to eliminate these effects. Finally, the technology of the magnetic source imaging software used in this study is limited; therefore, we need to use other magnetic source imaging software in follow-up research to validate these results.

Conclusion

This study investigated the relationship between magnetic brain activity and cognitive function in children with SeLECTS (SWI $\geq$ 50%) compared to SeLECTS (SWI < 50%) patients and healthy controls. We found a significant decrease in cognitive function in the SeLECTS (SWI $\geq$ 50%) group compared to the SeLECTS (SWI < 50%) and HC groups, and a significant difference in magnetic source activity between the three groups. Deactivation of magnetic source activity in the PCC and MFC regions was the main cause of cognitive function decline in SeLECTS patients and showed some frequency dependence. We intend to follow up with these patients and to clarify the prognosis of SeLECTS (SWI $\geq$ 50%) vs. SeLECTS (SWI < 50%) and the corresponding changes in magnetic brain activity.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by the Medical Ethics Committee of Nanjing Medical University, Nanjing Brain Hospital and Nanjing Children's Hospital. Written informed consent to participate in this study

was provided by the patients/participants' legal guardian/next of kin. Written informed consent was obtained from the minor(s)' legal guardian/next of kin for the publication of any potentially identifiable images or data included in this article.

Author contributions

YaL, YiL, JS, and XW designed the research. YaL, YX, KN, and PW recruited the participants and analyzed the data. YaL, YW, KZ, and QC acquired the images. XW revised the manuscript. All authors approved the final submitted version and agreed to be accountable for its content.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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The effect of interictal epileptic discharges and following spindles on motor sequence learning in epilepsy patients

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Purpose: Interictal epileptic discharges (IEDs) are known to affect cognitive function in patients with epilepsy, but the mechanism has not been elucidated. Sleep spindles appearing in synchronization with IEDs were recently demonstrated to impair memory consolidation in rat, but this has not been investigated in humans. On the other hand, the increase of sleep spindles at night after learning is positively correlated with amplified learning effects during sleep for motor sequence learning. In this study, we examined the effects of IEDs and IED-coupled spindles on motor sequence learning in patients with epilepsy, and clarified their pathological significance.

Materials and methods: Patients undergoing long-term video-electroencephalography (LT-VEEG) at our hospital from June 2019 to November 2021 and age-matched healthy subjects were recruited. Motor sequence learning consisting of a finger-tapping task was performed before bedtime and the next morning, and the improvement rate of performance was defined as the sleep-dependent learning effect. We searched for factors associated with the changes in learning effect observed between the periods of when antiseizure medications (ASMs) were withdrawn for LT-VEEG and when they were returned to usual doses after LT-VEEG.

Results: Excluding six patients who had epileptic seizures at night after learning, nine patients and 11 healthy subjects were included in the study. In the patient group, there was no significant learning effect when ASMs were withdrawn. The changes in learning effect of the patient group during ASM withdrawal were not correlated with changes in sleep duration or IED density; however, they were significantly negatively correlated with changes in IED-coupled spindle density.

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Conclusion: We found that the increase of IED-coupled spindles correlated with the decrease of sleep-dependent learning effects of procedural memory. Pathological IED-coupled sleep spindles could hinder memory consolidation, that is dependent on physiological sleep spindles, resulting in cognitive dysfunction in patients with epilepsy.

KEYWORDS

interictal epileptic discharge, spindle, focal epilepsy, motor sequence learning, non-rapid eye movement sleep

Introduction

Patients with epilepsy demonstrate chronic cognitive dysfunction, which is a major cause of quality of life impairment (1). Epileptic seizures, interictal epileptic discharges (IEDs), antiseizure medications (ASMs), psychiatric symptoms, and brain pathology are thought to be the causes of the cognitive dysfunction (2). IEDs are paroxysmal electroencephalographic findings and occur specifically in patients with epilepsy (3, 4). IEDs reflect the paroxysmal hypersynchronous firings of large neuronal populations, including normative neurons. Replacing the neuronal firings subserving physiological functions with the pathological ones at each IED could lead to transient cognitive dysfunction (5). Actually, there have been many reports about the instantaneous effects of IEDs during cognitive tasks (5–9). Generalized IEDs and IEDs with long duration are known to delay reaction time to stimuli regardless of the type and severity of epilepsy (10, 11). IEDs in the hippocampus and temporal lobe at the encoding and retrieval phase of the verbal memory tasks worsen recall performance in patients with drug-resistant focal epilepsy (12–15). Furthermore, recent studies indicate that IEDs may also have adverse effects on memory processing on a longer time scale, the consolidation of memory (16–18). IEDs during non-rapid eye movement (NREM) sleep particularly cause a decline of recall rate in patients with drug-resistant focal epilepsy (17), suggesting IEDs could deteriorate declarative memory consolidation by replacing neural activity involved in synaptic plasticity during NREM sleep. All of these studies were conducted using declarative memory tasks, and no study investigates the effect of IEDs on long-term memory using procedural memory tasks.

Sleep spindles are neural oscillations observed during NREM sleep with a frequency between 9 and 16 Hz and are associated with various cognitive functions. Especially, they have a central role in memory consolidation, in which synchronous activities across hippocampal sharp-wave ripples, sleep spindles, and cortical slow oscillations enhance hippocampal to cortex information transfer (19). Indeed, reduced coordination between sleep spindles and cortical slow oscillations correlates with impaired memory consolidation in patients with schizophrenia (20) and healthy elderly individuals (21). Among neural activities during NREM sleep, sleep spindles have often been investigated in relation to synaptic plasticity changes, particularly concerning procedural memory. Sleep spindle density increases after procedural memory tasks and the degree of increment correlates with the degree of improvement in performance (22, 23).

Recently, activities in the spindle frequency band following IEDs have been observed during NREM sleep in both rats and humans (24–26). The characteristics of individual IED-coupled spindles, such as duration, amplitude, frequency, and spatial distribution, are no different from physiological ones (24, 26). These findings indicate physiological and IED-coupled spindles share their generation mechanism. Accordingly, it has been hypothesized that IEDs disturb memory consolidation by inducing sleep spindles with inappropriate timing and replacing their physiological counterparts. Actually, these IED-coupled spindles have been reported to impair memory in rats (26) but there have been no reports that directly examine the effects of IED-coupled spindles on cognitive function in humans.

Motor sequence learning, classified as procedural memory, is one of the well-established tasks to study synaptic plasticity during sleep (27, 28). It exhibits a sleep-dependent learning effect, in which the content learned before sleep is enhanced by subsequent sleep without additional training and is attenuated by sleep deprivation, especially in NREM sleep (29, 30). This sleep-dependent learning effect positively correlates with spindle density in NREM sleep after learning (23). Only two studies have investigated sleep-dependent learning effect of patients with epilepsy to our knowledge. One compared the learning effect of epilepsy patients with that of healthy subjects; the former tended to be weaker than the latter, although the difference was not significant (31). The other study examined the impact of epileptic seizures on the learning effect of patients

Abbreviations: ASM, antiseizure medication; IED, interictal epileptic discharge; LT-VEEG, log-term video-encephalography; NREM, non-rapid eye movement.

with epilepsy, proposing epileptic seizures themselves could impair learning effect; however, the impact of IEDs was not investigated (32).

The aim of this study is to elucidate the mechanism by which IEDs cause cognitive dysfunction in patients with epilepsy. If the effects of IEDs on cognitive function are clarified in this study, IEDs themselves could be targeted for the treatment of chronic cognitive dysfunction in epileptic patients. We hypothesized that IEDs or IED-coupled spindles during NREM sleep could disrupt sleep-dependent learning effects of motor sequence learning by interfering with physiological neural activity involved in synaptic plasticity. To verify this hypothesis, we conducted motor sequence learning in patients with epilepsy and studied the relationship between results of the overnight electroencephalography (EEG) analysis and sleep-dependent learning effect.

Materials and methods

The present study was a prospective observational study with minor task intervention in patients with focal epilepsy who were treated at Kyushu University Hospital and age-matched healthy subjects, approved by the Kyushu University Institutional Review Board for Clinical Research (20192003). We attempted to corroborate the existence of sleep-dependent learning disability in patients with epilepsy by comparing their learning effect with healthy controls. Furthermore, we aimed to determine the effect of IEDs and IED-coupled spindles on learning disability by performing the same intervention to the same patient at two time points (Trials 1 and 2) with different IED densities, then analyzing the correlation between the degree of change in learning effect vs. that of IEDs and IED-coupled spindles.

Subjects selection

In the epilepsy patient group, patients between the ages of 18 and 75 years were included who were diagnosed with focal epilepsy and scheduled to undergo long-term video-electroencephalography (LT-VEEG) with ASM withdrawal at Kyushu University Hospital between June 1, 2019, and December 31, 2021 for clinical necessity. The validity of diagnosis was assessed by at least two expert epileptologists. We excluded patients who had been previously diagnosed with neurological or psychiatric disorders other than epilepsy, had undergone epilepsy surgery, had upper limb motor dysfunction, were professional musicians or typists, or worked at night.

In the healthy group, subjects between ages of 18 and 75 were included who were physically and mentally healthy and were not professional musicians, typists, nor night shift workers.

Task

The experiment was run in PsychoPy, an open-source experimental-control software package (33). A finger-tapping task was conducted in accordance with previous studies (34–36). Subjects were asked to type on a keyboard a sequence of five numbers from 1 to 4 (e.g., 4-1-3-2-4) with designated fingers of their non-dominant arm (1: index finger, 2: middle finger, 3: ring finger, 4: little finger). We prepared a desktop computer, displayed a digit sequence on the screen, and asked subjects to type the numbers shown on the screen. If they typed correctly, the bar displayed under the number moved to the next number, and if they typed incorrectly, the bar did not move and a warning buzzer sounded. Each block consisted of a 30-s typing period when subjects were instructed to type continuously the numbers as accurately and quickly as possible, followed by a 30-s break period when the subjects were asked to do nothing but stare at the center of the screen. Twelve blocks were performed during the training session before sleep, and three blocks were performed during the retest session the next morning. We prepared two types of digit sequences (4-1-3-2-4 and 2-3-1-4-2) and changed the sequences between Trials 1 and 2 for each subject. The sequence used for each trial was randomly and equally assigned among subjects to avoid bias. The number of digit sequences that were correctly typed for each block was added up, and the average sequence numbers correctly typed during the last three blocks of the training session were compared with those of the retest session (three blocks). The difference between the two sessions was divided by the average of the final three blocks of the training session, multiplied by 100, and expressed as a percentage; this was defined as the sleep-dependent learning effect in this study.

Overall design

The overall design of this study is shown in Figure 1. Since the measurability of IEDs depends on where the seizure onset zone is located (37), we decided to employ percentage differences for normalization by obtaining IED data of two distinct ASM statuses: the ASM withdrawal period when IEDs are expected to increase and the regular medication period when IEDs are expected to stabilize and diminish. Due to conducting the same learning task twice to a subject within a short timeframe, the potential for the skill and familiarity acquired at the first learning intervention causing a cross-learning effect for the second had to be considered. If there was a difference of learning effect between the two interventions, we could not establish whether the difference was attributed to the cross-learning effect or change in IEDs. Thus, we conducted the same interventions to the healthy group for control. Taking into consideration the minimum time needed to return to a steady state after

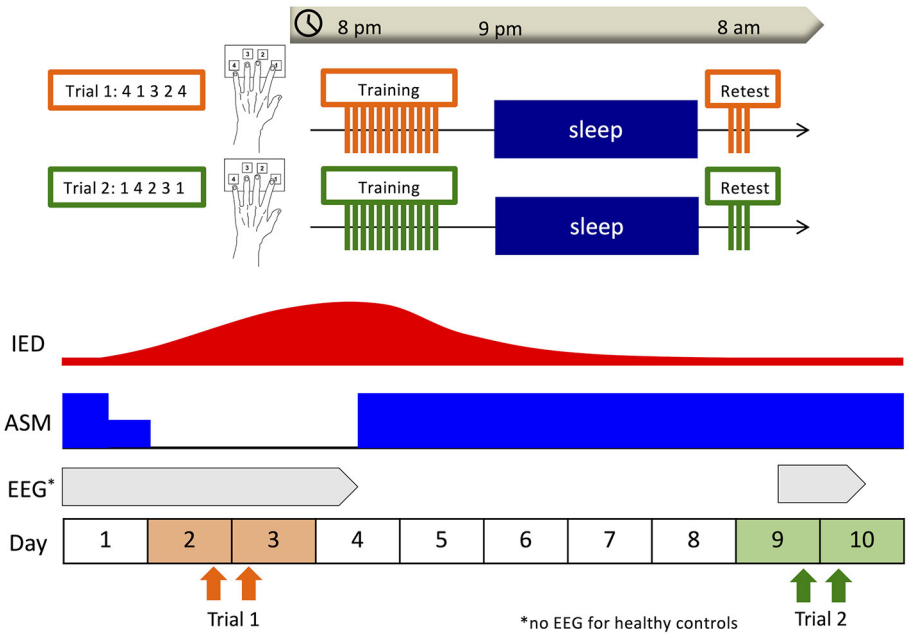

FIGURE 1
Overall design. Subjects underwent the training session of the finger-tapping task at 8:00 p.m., and the retest session at 8:00 a.m. the next morning. In the patient group, the first trial was conducted on the second night of hospitalization when antiseizure medications (ASMs) were withdrawn for clinical necessity (Trial 1), and the second trial was conducted on the ninth night of hospitalization after ASMs had been returned to usual doses (Trial 2). In the healthy control group, the start date of Trial 1 was set according to the subject's convenience, and Trial 2 was conducted 1 week later. In the patient group, overnight electroencephalography (EEG) was recorded including the nights after each training session as part of long-term video-EEG.

resuming usual doses of ASMs, the two learning interventions were conducted within a week of each other. This 1-week interval ensured the two interventions could be completed within the same hospital stay. In the healthy group, subjects visited our analysis room in the Kyushu University Hospital at 8:00 p.m., and after a medical interview and brief guidance they underwent the training session of a finger-tapping task. After the training session, the subjects were asked not to practice, recall the task, or use any electronic devices and instruments that required finger tapping, which might interfere with the consolidation of motor sequence learning, and to go to bed as usual. The next morning at 8:00 a.m., they underwent the retest session using the same digit sequence in our analysis room. For the patient group, we visited the patient's room at 8:00 p.m. and the next morning at 8:00 a.m. and administered the same intervention as the healthy subjects. The first trial was conducted on the second night of hospitalization when ASMs were temporarily withdrawn to induce seizures due to clinical necessity as part of LT-VEEG (Trial 1). The second trial was conducted on the ninth night of hospitalization after ASMs were returned to their usual doses (Trial 2). In the healthy control group, the start date of Trial 1 was set according to the subject's convenience, and Trial 2 was conducted 1 week later.

Data acquisition

In the patient group, an overnight EEG was recorded on the night after each training session as part of LT-VEEG using the Nihon Kohden Neurofax. Nineteen scalp electrodes (Fp1/Fp2/F3/F4/C3/C4/P3/P4/O1/O2/F7/F8/T3/T4/T5/T6/Fz/Cz/Pz) were placed according to the International 10–20 method. The average of C3 and C4 was employed as the system reference for recordings. Referential derivation (unipolar derivation) referring to the electrode placed on the ipsilateral mastoid was used to determine sleep stage and detect spindles, while referential derivation and multiple bipolar derivations were used to detect IEDs. Electrooculogram and electromyogram were recorded simultaneously with EEG. Overnight EEG was recorded in a private hospital ward room specialized for LT-VEEG and soundproofed with adequately controlled temperature. Electrode impedances were measured and dropped below 10 kΩ by skilled clinical neurophysiologists before recording. EEG recordings were constantly checked, and electrode detachment was promptly corrected. All scalp electrodes (Fp1/Fp2/F3/F4/C3/C4/P3/P4/O1/O2/F7/F8/T3/T4/T5/T6/Fz/Cz/Pz) were used to detect IEDs and determine the stage of sleep, and nine electrodes (F3/F4/C3/C4/P3/P4/Fz/Cz/Pz) were

used to detect spindles. The sampling frequency was 1 kHz. In line with the 2012 criteria of the American Academy of Sleep Medicine (AASM) (38), two expert electroencephalographers determined sleep stages for every 30-s epoch of EEG recordings from 8:00 p.m. to 8:00 a.m. the next morning. They visually identified spikes, sharp waves, spike-and-slow-wave complexes, sharp-and-slow-wave complexes, polyspike complexes, and polyspike-and-slow-wave complexes during NREM sleep as IEDs using standard amplitude and duration criteria (39). Specifically, a spike or sharp wave was recognized as a transient, clearly distinguished from background activity, with a pointed peak, amplitude larger than 50 μV, and duration between 20 and 70 ms (spike) or 70 and 200 ms (sharp wave). A sequence of two or more spikes was labeled a polyspike complex. They were modified as a spike-and-slow-wave complex, sharp-and-slow-wave complex, or a polyspike-and-slow-wave complex if an associated slow wave separate from background activity followed them. Differences in findings between the two electroencephalographers were reconciled later.

Spindle autodetection

All analyses of the acquired EEG signals were performed in MATLAB 2018b (The Mathworks Inc., Natick, MA, USA). Spindles were automatically detected in all NREM sleep recordings by the previously adopted algorithm in a study on epilepsy patients and model rats (26). In this algorithm that captures activity above a predefined threshold after normalization, contamination by artificial or pathological high-amplitude signals could precipitate the underestimation of spindles. Therefore, we excluded epochs containing artifacts or visually detected IEDs from the analysis and rejected all outliers before normalization, as described later. The raw data were downsampled to 256 Hz and then notch-filtered at 60 Hz and its multiples to remove the effects of alternating current. The downsampled data were first band-pass filtered from 1 to 50 Hz to extract broadband data, then band-pass filtered from 10 to 20 Hz to extract spindle-band data. After excluding outliers (exceeding the third quartile + 1.5*interquartile range), the power values of the amplitudes were extracted using the Hilbert transform and standardized for each electrode by its average value. For discerning spindle shape, we selected continuous activity above 1 standard deviation (SD) with a peak of more than 3 SDs and duration between 0.5 and 2 s as spindle candidates. To exclude false positives stemming from other frequencies, power spectrum analysis was performed on the broadband data within the timeframe of each detected spindle candidate, and those which maximum frequencies were between 11 and 16 Hz were extracted as spindles. These parameters follow a previous study detecting IED-coupled spindles (26). To validate the possible effects of IEDs on spindle detection, we calculated recall value, precision value, and F-score for each patient by comparing the spindles visually identified by a skilled electroencephalographer, independent from the analysis, with those detected by the algorithm (40). Then, we compared these parameters between the patient groups with and without IEDs. This validation was performed on the continuous 1-h sleep records in Trial 1 at C3 when epileptic activities were assumed to be the most influential due to the withdrawal of ASMs. Among the detected spindles, we defined those that began within 1 s after peak of IED as pathological spindles (24, 26). Pathological spindles were detected for each electrode, and the density of pathological spindles was calculated by dividing the number of spindles by the duration of NREM sleep.

Statistics

All statistical analyses were performed using JMP 14.0 statistical software (SAS Institute, Cary, NC). P-value < 0.05 was regarded as statistically significant in all statistical analyses. As epileptic seizures have been reported to impair sleep-dependent learning effects in motor sequence learning, patients who had seizures at the night after training sessions were excluded to focus purely on the effects of IEDs (32). The number of correctly-typed sequences per block was compared between the patient group and control group using a linear mixed model (LMM) for each trial. The number of correctly-typed sequences per block was assigned as the dependent variable; groups (epilepsy and control), blocks (12 training and three retest), and group*block interaction as fixed effects; and each subject as a random effect. The means of sleep-dependent learning effect for each group in each trial was compared to zero using the one-sample t-test. Moreover, to verify whether there was a difference in sleep-dependent learning effect between Trials 1 and 2 excluding the potential cross-learning effect due to acquired skill and familiarity at Trial 1 affecting learning effect at Trial 2, we compared the means of sleep-dependent learning effect between different groups and trials using a LMM. Sleep-dependent learning effect was set as the dependent variable; the groups (epilepsy and control), trials (Trials 1 and 2), and their interaction as fixed effects; and each subject as a random effect.

In the epilepsy group, we explored possible factors that could have had an association with the change in learning effect between Trials 1 and 2 using simple linear regression analysis with Bonferroni correction. Changes between Trials 1 and 2 in NREM sleep duration, IED density during NREM sleep, all spindle density during NREM sleep, and pathological spindle density during NREM sleep were assessed. The relationship in absolute values, not in changes, between these explanatory variables and the sleep-dependent learning effect was also evaluated separately for each trial. Since pathological spindles assume the presence of IEDs, patients whose EEG did not have discernable IEDs were excluded from regression analyses on IEDs and pathological spindles.

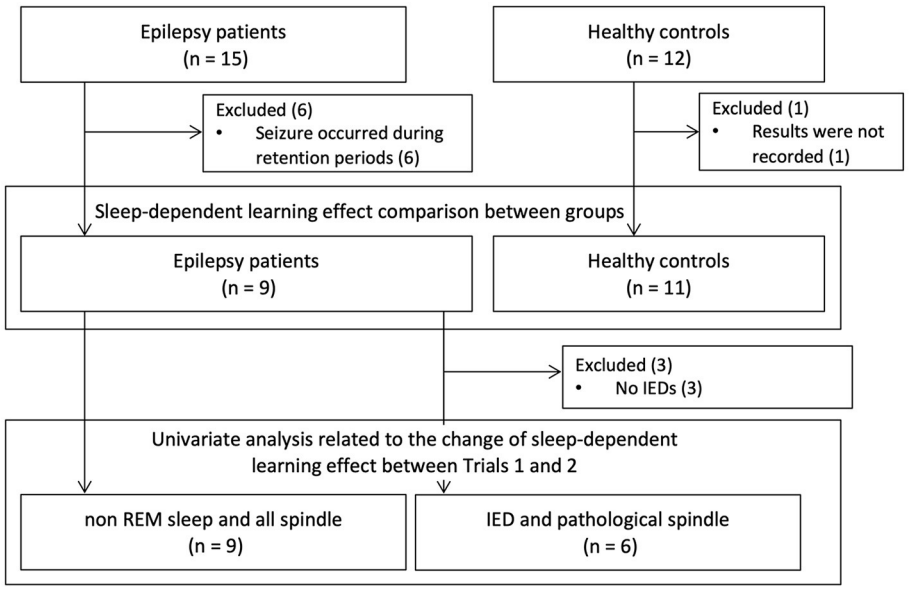

FIGURE 2
Study population. Fifteen patients with epilepsy and 12 healthy subjects were recruited. Excluding six patients who had epileptic seizures the night after training and one healthy subject whose recordings were defective, we compared sleep-dependent learning effects between nine patients and 11 healthy controls. Then, we exploratively analyzed the factors associated with changes in sleep dependent-learning effect between Trials 1 and 2. When we analyzed about IED and pathological spindle, we excluded three patients who did not have any IEDs. IED, interictal epileptic discharge.

Results

Demographics

The study population is shown in Figure 2. Fifteen patients with epilepsy and 12 healthy subjects were recruited. Six patients who had epileptic seizures the night after training sessions were excluded. We excluded one healthy subject who had an incomplete study record due to a problem with computer operation. We examined whether there was a difference in sleep-dependent learning effects between the nine epilepsy patients and 11 healthy subjects. We also analyzed the correlation with sleep-dependent learning effects in all the nine patients for NREM sleep duration and all detected spindle density, and in six patients, excluding a further three patients without IEDs (Patients 4, 6, and 9), for IED density and pathological spindle density. The demographics of the nine patients are shown in Supplementary Table 1. Mean age was 28.7 years (SD = 10.6 years) in the patient group and 30.2 years (SD = 10.8 years) in the healthy group, with no significant difference [$t_{(17.5)}$, $P = 0.76$]. All subjects in both the patient and healthy groups were right-handed. Age of onset, duration of illness, seizure focus, and ASM varied among patients. We visually detected IEDs in six patients (Patients 1, 2, 3, 5, 7, and 8). The mean IED density among the six patients was 3.3/min (SD = 4.5/min) in Trial 1 and 2.5/min (SD = 3.9/min) in Trial 2. Sleep variables for the patient group are shown in Supplementary Table 2. Upon withdrawal of ASMs, six patients (66.7%) experienced reduction in NREM sleep duration, and all six patients with IEDs exhibited a rise in IED density. When the means of these variables between Trials 1 and 2 were compared by the paired *t*-test, there was a significant difference in IED density [$t_{(5)} = -3.1$, $P = 0.028$], but not in sleep variables (Supplementary Table 2).

Motor sequence learning and its sleep-dependent learning effect

In Trial 1, a LMM for number of correctly typed sequences demonstrated the main effects of group [$F_{(1,18)} = 11.9$, $P = 0.0028$] and block [$F_{(14,252)} = 32.2$, $P < 0.0001$], and the interaction effect between them to be significant [$F_{(14,252)} = 2.3$, $P = 0.0052$]. A following *post-hoc* Tukey HSD test clarified that the number of correctly-typed sequences per block in the epilepsy group was significantly lower than in the control group for all blocks of the retest session as shown in Figure 3 [block 1: 13.1 (SD = 5.9) vs. 24.8 (SD = 7.6), $P = 0.013$; block 2: 15.3 (SD = 6.3) vs. 26.9 (SD = 7.4), $P = 0.015$; block 3: 28 (SD = 7.9) vs. 15.8 (SD = 5.2), $P = 0.0062$]. Although a LMM for Trial 2 showed significant main effects in groups [$F_{(1,18)} = 9.0$, $P = 0.0076$] and blocks [$F_{(14,252)} = 38.7$, $P < 0.001$], there was no significant interaction between them [$F_{(14,252)} = 1.3$, $P = 0.22$]. While none of the healthy subjects' performances

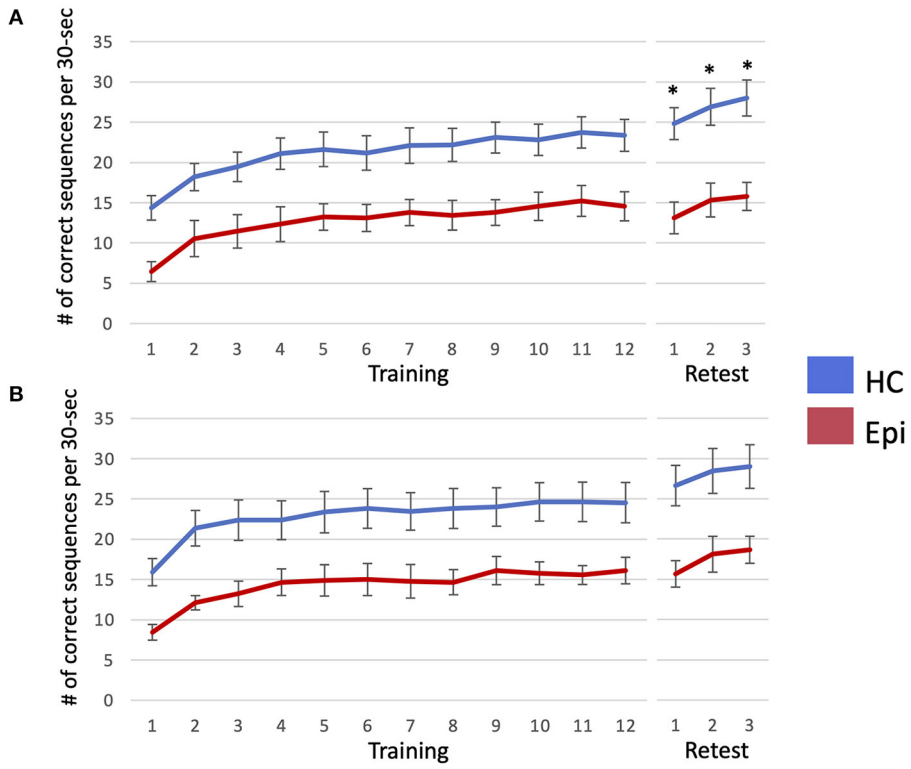

FIGURE 3
The number of correctly-typed sequences is plotted per each block of Trial 1 **(A)** and Trial 2 **(B)**. Error bar indicates standard error. When the number of correctly-typed sequences were compared between the two groups using a linear mixed model for each trial, that of the healthy control group was significantly higher than that of the epilepsy patient group in all blocks of the retest session in Trial 1, as marked by asterisks. HC, healthy controls; Epi, patients with epilepsy.

deteriorated after sleep, two patients (22.2%) in Trial 1 and two patients (22.2%) in Trial 2 had worsened results after sleep. In the healthy group, the mean sleep-dependent learning effect was 13.8% (95%CI = 10.2–17.5%) in Trial 1 and 14.9% (95%CI = 8.24–21.6%) in Trial 2 (Figure 4), and significantly >0 in both Trials 1 [$t_{(10)} = 8.4$, $P < 0.001$] and 2 [$t_{(10)} = 5.0$, $P = 0.006$]. In the patient group, the mean sleep-dependent learning effect was 1.0% (95%CI = −13.7–15.7%) in Trial 1 and 9.3% (95%CI = 1.42–17.3%) in Trial 2, and significantly greater zero in Trial 2 [$t_{(8)} = 2.7$, $P = 0.026$] but not Trial 1 [$t_{(8)} = 0.16$, $P = 0.87$]. A LMM for sleep-dependent learning effect discovered a significant main effect of group [$F_{(1,18)} = 4.8$, $P = 0.041$], but not of trial [$F_{(1,18)} = 2.0$, $P = 0.17$], and interaction between group and trial was not significant [$F_{(1,18)} = 1.2$, $P = 0.29$].

Pathological spindle

Spindles were automatically detected from nine electrodes (F3/F4/C3/C4/P3/P4/Fz/Cz/Pz) of each patient. Results validating the algorithm are shown in Supplementary Figure 1. An average value of recall among the nine patients was 0.81 (SD = 0.088), that of precision was 0.88 (SD = 0.074), and that of F-score was 0.85 (SD = 0.073). When we compared the means between patients with IEDs (patients 1, 2, 3, 5, 7, and 8) and those without (patients 4, 6, and 9) by student's *t*-test, there was no significant difference [recall: 0.78 (95% CI = 0.69–0.87) vs. 0.87 (95% CI = 0.73–1.0), $P = 0.11$; precision: 0.89 (95% CI = 0.80–0.97) vs. 0.87 (95% CI = 0.73–1.0), $P = 0.84$; F-score: 0.83 (95% CI = 0.75–0.92) vs. 0.87 (95% CI = 0.74–1.0), $P = 0.40$]. The topography of the mean power and density of all detected spindles including pathological spindles showed symmetric distribution dominant in the centro-parietal region (Supplementary Figure 2), consistent with previous reports (21, 41, 42). The density of all detected spindles had no correlation with age (Supplementary Table 3). Pathological spindles were detected in five of the six patients with IEDs (Patients 1, 2, 3, 5, and 8). Mean pathological spindle density among the five patients was 0.058/min (SD = 0.020/min) in Trial 1 and 0.050/min (SD = 0.017/min) in Trial 2. There was no significant difference between Trials 1 and 2 in the density of all detected spindles and that of pathological spindles for each channel (Supplementary Table 4). In contrast to IEDs, pathological spindles did not necessarily increase uniformly with ASM withdrawal; their pattern of change between Trials 1 and 2 varied among patients and electrodes (Figure 5).

Pathological spindles distributed differently among patients and did not overlap with the seizure onset zone.

The factors associated with sleep-dependent learning effect

We next explored the factors influencing sleep-dependent learning effects in the patient group (Table 1). Since changes in density of IEDs and pathological spindles showed Poisson distributions, they were converted to the square root. Changes between trials in duration of NREM sleep ($\beta = -0.19$, 95% CI $= -0.41$–0.042, $P = 0.10$), the density of IEDs during NREM sleep ($\beta = 8.1$, 95% CI $= -1.7$–17.9, $P = 0.082$), and the density of all detected spindle during NREM sleep (C4: $\beta = -9.8$, 95% CI $= -38.1$–18.5, $P = 1.0$; the details of other channels are shown in Table 1) were not associated with changes in sleep-dependent learning effect. Conversely, changes in the density of pathological spindles in C4 during NREM sleep had a significant negative correlation with changes in sleep-dependent learning effect ($\beta = -634$, 95% CI $= -858$ to -410, $P = 0.013$) (Figure 6). The results of other channels are shown in Table 1. When focusing on each trial, no significant direct relationship in absolute values was observed between sleep-dependent learning effect and these explanatory variables (Supplementary Table 5).

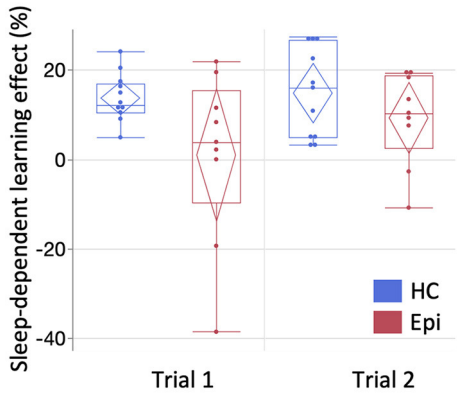

FIGURE 4
The comparison of sleep-dependent learning effect between groups. The mean sleep-dependent learning effect of the patient group was compared with the healthy group for each trial. Box-and-whisker plot is shown here. Starting from the bottom, each line represents the minimum, lower quartile, median, upper quartile, and maximum. Diamonds indicate the 95% confidence interval of each mean. One-sample *t*-test compared to zero showed significance in Trials 1 [$t_{(10)} = 8.4$, $P < 0.0001$] and 2 [$t_{(10)} = 5.0$, $P = 0.0006$] of the healthy group and in Trial 2 of the epilepsy group [$t_{(8)} = 2.7$, $P = 0.026$], but not in Trial 1 of the epilepsy group [$t_{(8)} = 0.16$, $P = 0.87$]. HC, healthy controls; Epi, epilepsy patients.

Discussion

The present study is one of the few studies that examine sleep-dependent learning effects of motor sequence learning in patients with epilepsy. We found that there was no significant sleep-dependent learning effect in epilepsy patients when ASM was withdrawn to augment epileptic activity. Furthermore, we found that the increase of IED-coupled spindles, rather than IEDs themselves, significantly correlated with the decrease of sleep-dependent learning effect. This is the first attempt to clarify the pathological significance of IED-coupled spindles in humans.

Sleep-dependent learning effect

Motor sequence learning is a procedural memory, and whether this kind of memory is impaired in patients with epilepsy has not been well investigated (43). Recently,

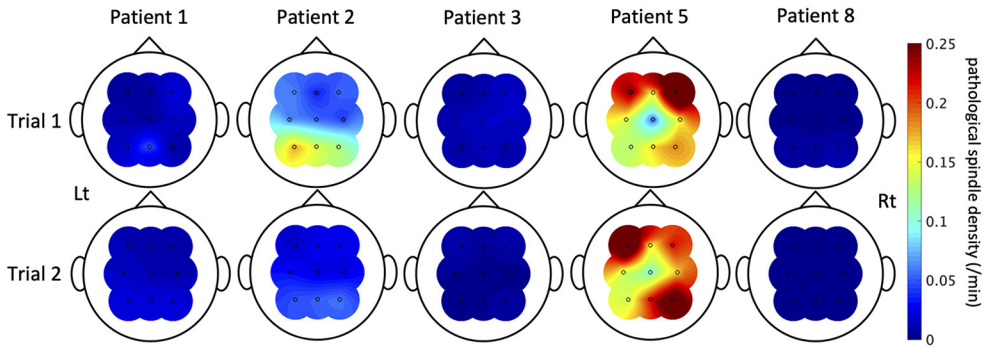

FIGURE 5
The pathological spindle density distributions. The distributions of interictal epileptic discharge (IED)-coupled spindles are shown here of the five patients in whom IED-coupled spindles were present. The color bar indicates the density of IED-coupled spindles. The nature of fluctuation of IED-coupled spindles between Trials 1 and 2 varied among patients and electrodes.

TABLE 1 Regression analysis with change in sleep-dependent learning effect (Trial 1 – Trial 2).

	β	95% CI	*P*
All patients (*N* = 9)			
Change of NREM sleep duration (Trial 1 – Trial 2)	−0.19	−0.41, 0.042	0.10
Change of all spindle density (Trial 1 – Trial 2)			
F3	4.8	−26.9, 36.4	1.0
F4	8	−29.4, 45.4	1.0
C3	−5.2	−35.6, 25.2	1.0
C4	−9.8	−38.1, 18.5	1.0
P3	−7.4	−27.5, 12.7	1.0
P4	−5.1	−21.0, 10.8	1.0
Fz	8.8	−35.6, 53.2	1.0
Cz	−2.8	−18.0, 12.3	1.0
Pz	−2.9	−27.6, 21.8	1.0
Patients with IEDs (*N* = 6)			
Change of IED density during NREM sleep (Trial 1 – Trial 2)	8.1	−1.7, 17.9	0.082
Change of pathological spindle density (Trial 1 – Trial 2)			
F3	−156	−267, −45.5	0.16
F4	101	−36.5, 238.7	1.0
C3	−284	−1,040, 473	1.0
C4	−634	−858, −410	***0.013***
P3	−85	−247, 77.0	1.0
P4	−123	−214, −32.5	0.18
Fz	−881	−2,885, 1,122	1.0
Cz	−276	−382, −171	***0.017***
Pz	−144	−255, −32.8	0.21

Simple regression analysis was conducted of differences between Trials 2 to 1 of sleep-dependent learning effect against of NREM sleep duration and all spindle density for all the nine patients, and IED density and pathological spindle density for the six patients with IEDs. The estimate (β), the 95% CI, and P-value are shown here. P-values were corrected by Bonferroni correction. $P < 0.05$ was considered significant and shown in bold italic.

it has become evident that its sleep-dependent learning effect, especially with regards to spatial component, depends on the hippocampus (44–46); patients with epilepsy with damage to the hippocampus could therefore develop impaired sleep-dependent learning effect. Although the impairment of hippocampus-mediated sleep-dependent memory consolidation has been investigated in the field of declarative memory for patients with focal epilepsy (16), there are still few reports in the field of procedural memory such as motor sequence learning. Deak et al. (31) compared the sleep-dependent learning effects of motor sequence learning between patients with temporal lobe epilepsy and healthy subjects. However, there was no significant difference in learning effect between groups, possibly due to the small number of cases. van Schalkwijk et al. (32) examined the effect of epileptic seizures on motor sequence learning in patients with focal epilepsy and found that sleep-dependent learning effect was reduced in the group with epileptic seizures. There has been no study, however, on IEDs in relation to sleep-dependent learning effect. ASMs could also affect cognitive function in epilepsy patients (47), but it is difficult to align ASMs across patients because ASMs are selected based on individual clinical backgrounds. Therefore, we performed two separate interventions in the same patient, one during ASM withdrawal and the other during usual doses of ASMs, and compared results between the two interventions against controls using a LMM. Nevertheless, the LMM showed no significant interaction between groups and trials in the present study. Low statistical power may be a possible reason for this because when the number of correctly-typed sequences was compared for each trial, the healthy control group showed higher scores than the patient group in the retest session only in Trial 1 (Figure 3A). Furthermore, we found that patients with epilepsy did not display any significant sleep-dependent learning effect only when ASMs were withdrawn. These results suggest that ASM withdrawal and ensuing enhanced IEDs, rather than the disease itself, could affect the learning effect. In this study, we found a significant correlation between changes in learning effect and those in IED-coupled spindle density. However, because IED-coupled spindles can be affected by age, ASM variety (48) and epilepsy type (49), larger sample size experiments controlling for these factors are needed to discuss the causal relationship between IED-coupled spindles and sleep-dependent learning effect.

Pathological spindles

In an exploratory analysis of factors associated with the changes in sleep-dependent learning effects of motor sequence learning, we found a significant correlation not with the density of IEDs *per se*, but with the density of IED-coupled spindles. This is the first report identifying the pathological significance of IED-coupled spindles in humans. A report that examined the pathological significance of IED-coupled spindles in model rats with temporal lobe epilepsy found an increase in IED-coupled spindles exacerbates memory consolidation impairment (26). The present clinical study affirmed exactly the same results in humans.

Spindles are formed in thalamic reticular nucleus neurons and project to the cortex *via* thalamocortical neurons (50). In NREM sleep, neocortical neurons fire synchronously with the Up state of slow oscillation, which stimulates pacemaker cells in the thalamic reticular nucleus to form a spindle in the thalamus and return it to the neocortex *via* thalamocortical neurons (51–53). These rhythmic and synchronous neural firings consistent with the spindle lead to efficient synaptic excitation of cortical neurons, shaping

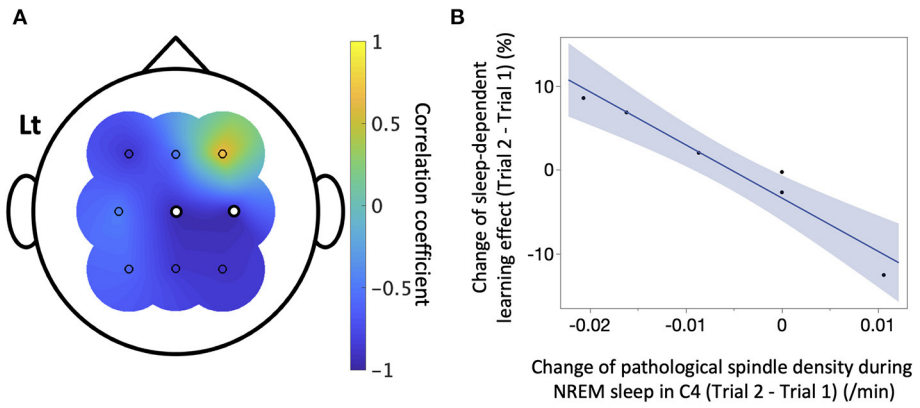

FIGURE 6
The correlation between sleep-dependent learning effect and pathological spindles. In the six patients with interictal epileptic discharges (IEDs), a simple linear regression analysis was conducted between the change between Trials 1 and 2 of sleep-dependent learning effect and that of IED-coupled spindle density of each electrode. **(A)** The correlation coefficient is shown here. There was a significant negative linear correlation in C4 ($\beta = -634$, 95%CI = −858 to −410, $P = 0.019$), marked as white dots. **(B)** A scatter plot of the result for C4 is displayed.

synaptic plasticity (52). Hippocampal ripples nesting in the slow oscillation-coupled spindles lead to active consolidation of memory *via* hippocampal-thalamic-neocortical synchronization (19). This synchronization among the three regions is essential for consolidating memory, and its artificial desynchronization impairs memory in rats (26, 54). Furthermore, spindles are perceived to have a labeling effect to assist subsequent rescaling by slow-wave sleep, in which the synchrony of spindles with the hippocampus and neocortex determines whether the labeled memory will undergo long-term potentiation or long-term depression (55–60). Although most of these findings relate to declarative memory, findings on procedural memory have been accumulating mainly in the field of schizophrenia in recent years. In patients with schizophrenia, the synchronization between spindles and slow oscillations was impaired and its degree associated with the sleep-dependent learning effect of motor sequence learning (61, 62). Moreover, synchronization among hippocampal ripples, spindles and slow oscillations was impaired in a mouse model of schizophrenia (20). Although there have been no studies directly analyzing the relationship between hippocampal ripples and procedural memory, it has been found that the sleep-dependent learning effect of motor sequence learning is impaired in subjects with lesions in the hippocampus (46). These findings suggest that synchronization among hippocampal ripples, thalamic spindles, and neocortical slow oscillations is important for memory consolidation in procedural memory as well as in declarative memory (63–65).

On the other hand, IEDs consist of sudden hypersynchronous firings initiated by the paroxysmal depolarization shifts of pathological cortical neurons and are followed by inhibitory postsynaptic potentials (3). Since direct electrical stimulation of the localized cortex (54, 66), depolarization of specific neurons by optogenetic techniques (55), as well as transcranial electrical and magnetic stimulation (67) can induce cortical spindles, the synchronous cortical firing by IEDs could also induce spindles in the thalamus *via* thalamocortical neurons (26). However, the erratic nature and timing of spindle formation induced by paroxysmal IEDs would mean they would not be synchronized with neocortical slow oscillations or hippocampal ripples. This could not only interfere with physiological memory consolidation but also promote eliminatory labeling which might debilitate spindle-dependent physiological synaptic plasticity (42, 68–70). Patients in this study who demonstrated an attenuation of sleep-dependent learning effect support this hypothesis. In this study, the harmful effect of IED-coupled spindles was most evident near the motor cortex of the intervening limb (i.e., C4), where the positive impact of physiological spindles on sleep-dependent motor learning is most significant (71–73). This finding might indicate that the replacement of physiological spindles with IED-coupled spindles associated with learning disabilities occur in a region-specific manner, but the confirmation by the further studies with larger sample size are required.

Limitations

There are several limitations in this study. Firstly, ASMs were not uniform among patients because the baseline ASMs and ASMs to be withdrawn were decided based on clinical necessity. Secondly, the number of cases was small, forcing sample ages and epilepsy types to be heterogeneous, because

the study was conducted during the prevalence of COVID-19. The number of patients included in the regression analysis on IED-coupled spindles was further limited to six, leading to low reliability. A study with a larger number of patients with comparable ASMs and ages is desirable. Lastly, because many IEDs and spindles could only be detected by intracranial recordings, we might have underestimated IEDs and IED-coupled spindles. In the future, we hope to perform similar interventions on patients with intracranial electrodes.

Conclusion

We found that patients with epilepsy showed no significant sleep-dependent learning effect of procedural memory during the ASM withdrawal period. Furthermore, we identified that the decrease of sleep-dependent learning effect significantly correlated with the increase of IED-coupled spindles and not the IEDs themselves. This is the first attempt to elucidate the pathological significance of IED-coupled spindles in humans.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving human participants were reviewed and approved by the Kyushu University Institutional Review Board for Clinical Research (20192003). The patients/participants provided their written informed consent to participate in this study.

Author contributions

TO and TU have contributed to the conception and design of the study. TO, TY, TM, AS, KO, HS, and TU have contributed to the acquisition and analysis of data. TO, HS, NI, and TU have contributed to the drafting and revising the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2022.979333/full#supplementary-material

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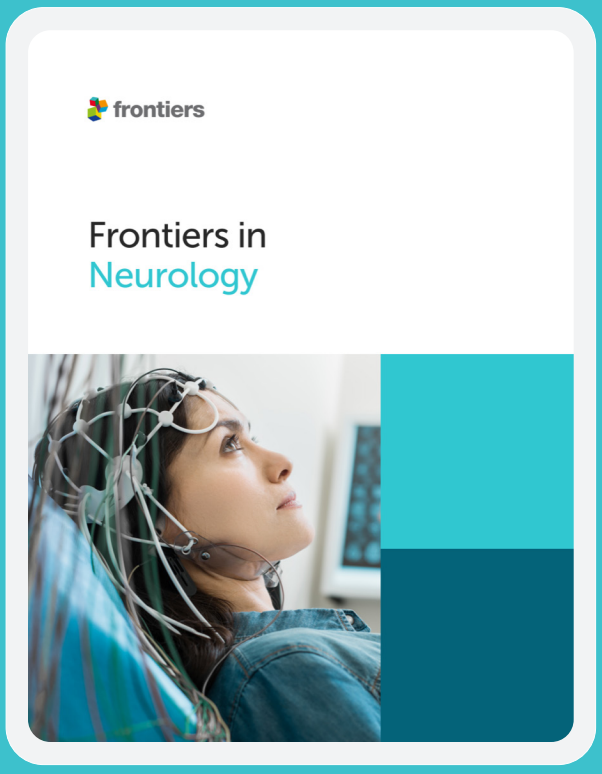

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