Structural network topology in limbic encephalitis is associated with amygdala enlargement, memory performance and serostatus

Tobias Bauer1 | Bastian David1 | Leon Ernst1 | Albert J. Becker2 | Juri-Alexander Witt1 | Christoph Helmstaedter1 | Jan Wagner3 | Bernd Weber4 | Christian E. Elger1 | Rainer Surges1 | Theodor Rüber1,5,6

1Department of Epileptology, University Hospital Bonn, Germany
2Department of Neuropathology, University Hospital Bonn, Bonn, Germany
3Department of Neurology, University of Ulm and Universitäts- and Rehabilitationskliniken, Ulm, Germany
4Institute of Experimental Epileptology and Cognition Research, University Hospital Bonn, Bonn, Germany
5Department of Neurology, Epilepsy Center Frankfurt Rhine-Main, Goethe University Frankfurt, Frankfurt am Main, Germany
6Center for Personalized Translational Epilepsy Research, Goethe University Frankfurt, Frankfurt am Main, Germany

Correspondence
Theodor Rüber, Department of Epileptology, University Hospital Bonn, Venusberg-Campus 1, 53127 Bonn, Germany.
Email: theodor.rueber@ukbonn.de

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Abstract
Limbic encephalitis (LE) forms a spectrum of autoimmune diseases involving temporal lobe epilepsy and memory impairment. Imaging features of LE are known to depend on the associated antibody and to occur on the brain network level. However, first studies investigating brain networks in LE have either focused on one distinct antibody subgroup or on distinct anatomical regions. In this study, brain graphs of 17 LE patients with autoantibodies against glutamic acid decarboxylase 65 (GAD-LE), four LE patients with autoantibodies against leucine-rich glioma-inactivated 1, five LE patients with autoantibodies against contactin-associated protein-like 2, 26 age- and gender-matched healthy control subjects, and 20 epilepsy control patients with hippocampal sclerosis were constructed based on T1-weighted structural magnetic resonance imaging scans and diffusion tensor imaging. GAD-LE showed significantly altered global network topology in terms of integration and segregation as compared to healthy controls and patients with hippocampal sclerosis (P < .01, analysis of variance with Tukey-Kramer post hoc tests). Linear regression linked global network measures with amygdala volume and verbal memory performance (P < .05). Alterations of local network topology show serotype dependence in hippocampus, amygdala, insula, and various cortical regions. Our findings reveal serotype-dependent patterns of structural connectivity and prove the relevance of in silico network measures on clinical grounds.

KEYWORDS
brain graph, limbic encephalitis, structural connectivity, temporal lobe epilepsy
INTRODUCTION

Limbic encephalitis (LE) comprises a spectrum of autoimmune brain diseases characterized by temporal lobe epilepsy and memory impairment.\(^1\) Growing evidence suggests that the clinical picture of LE patients including their magnetic resonance imaging (MRI) signature largely depends on the associated antibody.\(^2,3\) Antibodies against glutamic acid decarboxylase 65 (GAD), leucine-rich glioma-inactivated 1 (LGI1), and contactin-associated protein-like 2 (CASPR2) are most commonly found in LE patients.\(^1\) Previous imaging research in the field of LE has mostly focused on identifying structural alterations of either mesiotemporal gray matter regions or white matter tracts.\(^2,3\) Nevertheless, these approaches are limited in their ability to gain insights into pathological processes at a larger scale. To integrate previous findings into a global and functionally relevant framework, we chose to apply a structural connectivity network model.\(^8\) In this framework, we investigated alterations of network topology across multiple scales and serogroups. Moreover, we aimed to establish a link between in silico measures of network topology and well-established clinical parameters in the field of LE, in particular mesiotemporal volumetry and memory performance. To discriminate structural network topology between LE patients and other patients with focal epilepsy, we include epilepsy patients with hippocampal sclerosis (HS) as a clinical control group. To draw a complete picture, we opted to retain LE patient groups with only few subjects, although this requires a less powerful nonparametric approach for group-level statistical comparisons.

MATERIALS AND METHODS

2.1 Study group

Three experimental and two control groups were contrasted. For the experimental groups, we prospectively included 17 patients with GAD-autoantibody–associated LE (GAD-LE), four patients with LGI1-autoantibody–associated LE (LGI1-LE), and five patients with CASPR2-autoantibody–associated LE (CASPR2-LE). All LE patients were diagnosed according to widely acknowledged diagnostic criteria and harbored serologically proven autoantibodies.\(^9\) As it remains unclear whether LE is a predominantly unihemispheric process, we defined either one hemisphere as the predominantly affected hemisphere or both hemispheres as affected hemispheres according to a two-step classification scheme based on electroencephalographic data and mesiotemporal volumetry described elsewhere.\(^2,3,10\) Twenty-six healthy control subjects from a preexisting in-house database were individually age- and gender-matched to the LE patients. Moreover, presurgical scans of 20 epilepsy patients with histologically confirmed HS who underwent selective amygdalohippocampectomy were included. In all patients, figural and verbal memory performance was assessed.\(^11,12\) Memory parameters were standardized according to a conormalization sample of 488 healthy volunteers (mean = 100, SD = 10), applying a correction for age. See Table 1 and Supplementary material S1 for details. The study was approved by the internal review board of the University Hospital Bonn. All participants provided written informed consent.

| Group | GAD-LE | LGI1-LE | CASPR2-LE | HS | Controls | ANOVA, \(P\) |
|-------|--------|---------|-----------|----|----------|------------|
| n     | 17     | 4       | 5         | 20 | 26       | NA         |
| Female, n (%) | 10 (58.8) | 2 (50.0) | 0         | 11 (55.0) | 12 (46.2) | .47        |
| Lateralization, right/ bilateral/left | 4/5/8 | 2/1/1 | 1/2/2 | 4/0/16 | NA | .25 |
| Age, y | 35.50 ± 11.72 | 63.37 ± 4.90 | 57.35 ± 13.57 | 38.84 ± 13.22 | 44.63 ± 15.62 | <.001 |
| Age at onset, y | 31.75 ± 11.89 | 61.44 ± 4.60 | 54.39 ± 2.96 | 14.49 ± 11.86 | NA | <.001 |
| Disease duration, y | 3.75 ± 2.56 | 1.92 ± 2.09 | 2.96 ± 1.31 | 24.35 ± 14.27 | NA | <.001 |
| Verbal memory | 98.04 ± 11.35 | 80.31 ± 11.68 | 88.81 ± 6.00 | 84.26 ± 10.28 | NA | .002 |
| Figural memory | 87.04 ± 14.97 | 74.33 ± 7.66 | 91.89 ± 11.06 | 83.42 ± 61.81 | NA | .23 |
| Seizure-free at study, n (%) | 5 (29.4) | 3 (75.0) | 2 (40.0) | 1 (5.0) | NA | .014 |

Note: Continuous data are given as arithmetic group means ± standard deviation. \(P\) values correspond to analyses of variance for continuous data or Pearson chi-square tests for categorical data. Verbal memory test: Verbal Learning and Memory Test\(^12\); figural memory test: Diagnosticum für Cerebralschädigung, revised\(^11\); memory parameters were standardized according to a conormalization sample of 488 healthy volunteers (mean = 100, standard deviation = 10), applying a correction for age.

ANOVA, analysis of variance; CASPR2-LE, CASPR2-autoantibody–associated LE; GAD-LE, GAD-autoantibody–associated LE; HS, hippocampal sclerosis; LE, limbic encephalitis; LGI1-LE, LGI1-autoantibody–associated LE; NA, not applicable.
2.2 | Image acquisition

All subjects underwent diffusion tensor imaging with 60 directions and T1-weighted structural imaging in Bonn using a 3T MRI scanner (Magnetom Trio, Siemens Healthineers). Due to a scanner update in 2014, two different acquisition protocols and head coils were used. Details on acquisition parameters can be found in Supplementary Data S2. We accounted for this factor by introducing scanner update as an independent variable in the statistical models.

2.3 | Image preprocessing and network construction

Surface reconstruction and volumetric segmentation of T1 structural images were performed using FreeSurfer (v6.0, https://surfer.nmr.mgh.harvard.edu/); 84 cortical and subcortical regions of interest (ROIs) of the Desikan-Killiany atlas were used as nodes in the structural connectivity graph. Preprocessing of diffusion-weighted images was performed as described elsewhere3; streamline and network reconstruction was performed using the MRtrix3 toolbox (http://www.mrtrix.org). Edge weights were derived as the streamline count between each pair of ROIs and normalized to [0,1] on an intrasubject level.

2.4 | Global network analysis

Using the Brain Connectivity Toolbox, the clustering coefficient as a measure of segregation and the characteristic path length as a measure of integration were calculated for all individuals.8 Statistical comparisons between groups were performed in two steps. First, we tested for differences between GAD-LE, HS, and healthy controls using a one-way analysis of variance adjusting for age and scanner update with post hoc Tukey-Kramer pairwise tests. Second, to include LGI1-LE and CASPR2-LE groups, we tested for differences across all groups using a Kruskal-Wallis test with post hoc Dunn pairwise tests. A Benjamini-Hochberg procedure was applied to correct for multiple comparisons. Furthermore, regression analyses to predict amygdala volumes and memory performance based on global network measures across all LE serogroups were performed using linear regression including scanner update and individual intercepts for each serogroup as independent variables.

2.5 | Local network analysis

Differences in node strength were tested for every node across all groups using Kruskal-Wallis tests with post hoc Dunn pairwise tests and Benjamini-Hochberg correction for multiple comparisons. In the healthy control group, node strengths were pooled across both hemispheres. Unpaired t tests adjusting for age and scanner update were used to test for differences of connection weights between groups by means of network-based statistics including nonparametric permutation testing with 5000 permutations to correct for familywise error rate (FWE).13

3 | RESULTS

3.1 | Global network topology

An analysis of variance revealed significant group differences between GAD-LE, HS patients, and healthy controls in clustering coefficient ($F_{4,58} = 4.98, P = .002$) and characteristic path length ($F_{4,58} = 3.66, P = .010$). A Kruskal-Wallis test confirmed significant group differences across all groups in clustering coefficient ($\chi^2_4 = 15.78, P = .003$) and characteristic path length ($\chi^2_4 = 10.83, P = .03$). Post hoc pairwise tests revealed significantly lower clustering and a higher characteristic path length in GAD-LE as compared to HS patients and healthy controls at $P < .05$. By means of linear regression, the amygdala volume on the predominantly affected hemisphere ($F_{4,21} = 3.09, P = .038$, $R^2 = .31$) and verbal memory performance ($F_{4,21} = 4.39, P = .010, R^2 = .45$) were predicted by the clustering coefficient. The amygdala volume on the unaffected hemisphere, the amygdala volume averaged across both hemispheres, and figural memory performance were not significantly predicted by either clustering coefficient or characteristic path length at $P < .05$ (see Figure 1A,B and Figure S3).

3.2 | Local network topology

Comparing connection weights between GAD-LE and healthy controls, the network-based statistics approach detected 11 connections with significantly (FWE-corrected $P < .05$) lower weights across both hemispheres. When contrasting HS patients and healthy controls, only the connection between both lateral orbitofrontal gyri showed significantly lower weight in HS patients than healthy controls. All other contrasts did not reveal any significantly altered connections at FWE-corrected $P < .05$ (see Figure 1B and Figure S3B). Comparing node strengths across all groups, global Kruskal-Wallis tests revealed significant (false discovery rate–corrected $P < .05$) group differences for 18 nodes, including hippocampus, amygdala, and insula (Figure 1C and Figure S4C).
In the present study, we describe altered network topology in three distinct LE serogroups and aim to relate our findings to their clinical and cognitive profiles. Although the interpretation of altered network topology in a clinical context remains challenging, mesiotemporal volumetry is a clinically recognized marker for diagnostics and follow-up monitoring in the field of autoimmune LE. In this study, we show that lower clustering in LE patients is associated with greater amygdala enlargement in the predominantly affected hemisphere. Beyond imaging characteristics, memory impairment ranks among the most relevant cognitive deficits associated with LE. Although figural memory was independent of network topology, lower clustering was predictive for verbal memory impairment. This finding confirms a previously reported association between disease severity and verbal memory performance, which was related to structural integrity of the left hippocampus. This association was not found for figural memory performance.

With regard to local network topology, our results reflect previously described reduced interhippocampal and hippocampocortical functional connectivity in LGI1-LE. This
is further backed by our data regarding reduced hippocampal node centrality in LGI1-LE and GAD-LE. However, our finding of reduced insular node centrality in GAD-LE and LGI1-LE is novel and supports previous reports indicating a possible involvement of the insula in GAD-LE and LGI1-LE.16,17 Nevertheless, the pathomechanism of this finding remains a topic for future study. In comparison to HS patients, GAD-LE shows more widespread alterations of local network topology. Whereas in HS patients reduced node strengths are restricted to the predominantly affected hippocampus and both parahippocampal gyri, we found 18 anatomical regions with reduced node strength across both hemispheres in GAD-LE. Notably, in 10 nodes this reduction is significant in comparison to HS patients. In contrast to HS patients, reduced node strengths as well as reduced connection weights in GAD-LE largely cover bilateral subcortical gray matter regions, which may be associated with previously reported basal ganglia hypermetabolism in these patients.18

Altogether, our findings underline the concept that GAD-LE is a disease entity involving a variety of anatomical structures across the entire brain and beyond the mesial temporal lobe.3,5 With regard to LGI1-LE and as previously hypothesized, in contrast, our results point toward a less widespread pathology with a clear focus on mesiotemporal structures.5,6 The absence of significant alterations of network topology in CASPR2-LE may be grounded in insufficient statistical power due to the small sample size on the one hand, and on the other hand, previous literature has reported only subtle MRI findings in CASPR2-LE.6

Three aspects limit the explanatory power of the present study. First, small sample sizes in the LGI1-LE and CASPR2-LE groups made the use of low-power nonparametric statistical approaches unavoidable. Moderate group-level effects might therefore become apparent with increasing statistical power in a larger study cohort. Second, transversal study designs like the present one are challenged by therapeutically induced alterations of network topology in the LGI1-LE group. Third and last, the biological underpinnings of altered network topology remain speculative and require further investigation. Nonetheless, this study pioneers in linking observed network alterations across different scales and serological groups to well-established clinical parameters in the field of LE.

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CONFLICT OF INTEREST

C.E.E. has received fees as a speaker or consultant from UCB Pharma, Desitin, Bial, and Eisai. R.S. has received fees as a speaker or consultant from Bial, Cyberonics, Desitin, Eisai, LivaNova, Novartis, and UCB Pharma. None of the other authors has any conflict of interest to disclose.

ETHICAL PUBLICATION STATEMENT

We confirm that we have read the Journal’s position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

ORCID

Tobias Bauer https://orcid.org/0000-0002-0555-6214
Bastian David https://orcid.org/0000-0002-0146-0629
Leon Ernst https://orcid.org/0000-0001-8146-4234
Albert J. Becker https://orcid.org/0000-0003-2661-3705
Juri-Alexander Witt https://orcid.org/0000-0001-5640-2592
Christoph Helmstaedter https://orcid.org/0000-0002-6608-6244
Jan Wagner https://orcid.org/0000-0002-0459-8885
Bernd Weber https://orcid.org/0000-0002-7811-9605
Christian E. Elger https://orcid.org/0000-0002-2531-6701
Rainer Surges https://orcid.org/0000-0002-3177-8582
Theodor Rüber https://orcid.org/0000-0002-6180-7671

REFERENCES

1. Dubey D, Pittock SJ, Kelly CR, McKeon A, Lopez-Chiriboga AS, Lennon VA, et al. Autoimmune encephalitis epidemiology and a comparison to infectious encephalitis. Ann Neurol. 2018;83(1):166–77.
2. Ernst L, David B, Gaubatz J, Domínguez-Narciso I, Lüchters G, Becker AJ, et al. Volumetry of mesiotemporal structures reflects serostatus in patients with limbic encephalitis. Am J Neuroradiol. 2019;40(12):2081–9.
3. Bauer T, Ernst L, David B, Becker AJ, Wagner J, Witt J-A, et al. Fixel-based analysis links white matter characteristics, serostatus and clinical features in limbic encephalitis. Neuroimage Clin. 2020;27:102289.
4. Wagner J, Witt J-A, Helmstaedter C, Malter MP, Weber B, Elger CE. Automated volumetry of the mesiotemporal structures in antibody-associated limbic encephalitis. J Neurol Neurosurg Psychiatry. 2015;86(7):735–42.
5. Wagner J, Schoene-Bake JC, Witt JA, Helmstaedter C, Malter MP, Stoeker W, et al. Distinct white matter integrity in glutamic acid decarboxylase and voltage-gated potassium channel-complex antibody-associated limbic encephalitis. Epilepsia. 2016;57(3):475–83.
6. Heine J, Prüss H, Bartsch T, Ploner CJ, Paul F, Finke C. Imaging of autoimmune encephalitis—relevance for clinical practice and hippocampal function. Neuroscience. 2015;309:68–83.

7. Bien CG, Bien CI, Dogan Onugoren M, De Simoni D, Eigler V, Haensch C-A, et al. Routine diagnostics for neural antibodies, clinical correlates, treatment and functional outcome. J Neurol. 2020;267(7):2101–14.

8. Rubinov M, Sporns O. Complex network measures of brain connectivity: uses and interpretations. Neuroimage. 2010;52(3):1059–69.

9. Graus F, Titulaer MJ, Balu R, Benseler S, Bien CG, Cellucci T, et al. A clinical approach to diagnosis of autoimmune encephalitis. Lancet Neurol. 2016;15(4):391–404.

10. Navarro V, Kas A, Apartis E, Chami L, Rogemond V, Levy P, et al. Motor cortex and hippocampus are the two main cortical targets in LGI1-antibody encephalitis. Brain. 2016;139(4):1079–93.

11. Helmstaedter C, Pohl C, Hufnagel A, Elger CE. Visual learning deficits in nonresected patients with right temporal lobe epilepsy. Cortex. 1991;27(4):547–55.

12. Helmstaedter C, Durwen HF. The Verbal Learning and Retention Test. A useful and differentiated tool in evaluating verbal memory performance. Schweiz Arch Neurol Psychiat (1985). 1990;141(1):21–30. [in German].

13. Zalesky A, Fornito A, Bullmore ET. Network-based statistic: identifying differences in brain networks. Neuroimage. 2010;53(4):1197–207.

14. Loane C, Argyropoulos GPD, Roca-Fernández A, Lage C, Sheerin F, Ahmed S, et al. Hippocampal network abnormalities explain amnesia after VGKCC-Ab related autoimmune limbic encephalitis. J Neurol Neurosurg Psychiatry. 2019;90:965–74.

15. Heine J, Prüss H, Kopp UA, Wegner F, Then Bergh F, Münte T, et al. Beyond the limbic system: disruption and functional compensation of large-scale brain networks in patients with anti-LGI1 encephalitis. J Neurol Neurosurg Psychiatry. 2018;89(11):1191–9.

16. Bose G, Zwicker JC, Sitwell LD, Osman N, Fantaneau TA. Anti-LGI1 limbic encephalitis presenting as an expanding insular lesion. Can J Neurol Sci. 2019;46(6):770–2.

17. Falip M, Rodríguez-Bel L, Castañer S, Sala-Padró J, Miro J, Jaraba S, et al. Hippocampus and insula are targets in epileptic patients with glutamic acid decarboxylase antibodies. Front Neurol. 2019;9:1143.

18. Tripathi M, Tripathi M, Roy SG, Parida GK, Ihtisham K, Dash D, et al. Metabolic topography of autoimmune non-paraneoplastic encephalitis. Neuroradiology. 2018;60(2):189–98.

SUPPORTING INFORMATION
Additional supporting information may be found online in the Supporting Information section.

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