Functional connectivity of large-scale brain networks in patients with anti-NMDA receptor encephalitis: an observational study

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Summary

Background In anti-NMDA receptor (NMDAR) encephalitis, antibody-mediated dysfunction of NMDARs causes severe neuropsychiatric symptoms, including psychosis, memory deficits, and movement disorders. However, it remains elusive how antibody-mediated NMDAR dysfunction leads to these symptoms, and whether the symptoms arise from impairment in specific brain regions and the interactions between impaired regions.

Methods In this observational study, we recruited 43 patients with anti-NMDAR encephalitis from a tertiary university hospital and 43 age-matched and sex-matched healthy controls without a history of neurological or psychiatric disorders, who were recruited from the general population of Berlin. We used structural and resting-state functional MRI to investigate alterations in connectivity in all participants. We did functional connectivity analyses, including large-scale network analysis, whole-brain pair-wise connectivity, and machine-learning classification, and compared the results with patients' functional impairment.

Findings Although structural MRI was normal in 31 (72%) of the 43 patients, we observed widespread alterations of functional connectivity that correlated with clinical measures. These alterations included impaired hippocampal functional connectivity, decoupling of the medial temporal and the default-mode networks, and an overall impairment of frontotemporal connections. Furthermore, functional connectivity was impaired within distributed large-scale networks, including sensorimotor, frontoparietal, lateral-temporal, and visual networks. Memory impairment correlated with hippocampal and medial-temporal-lobe network connectivity, whereas schizophrenia-like symptoms were associated with functional connectivity changes in frontoparietal networks. Machine-learning analyses corroborated these findings and identified frontoparietal and frontotemporal connections as reliably discriminating features between patients and controls, yielding an overall accuracy of 81%.

Interpretation This study reveals a characteristic pattern of whole-brain functional connectivity alterations in anti-NMDAR encephalitis that is well suited to explain the major clinical symptoms of the disorder. These observations advance the pathophysiological understanding of NMDAR dysfunction in the human brain and could be similarly relevant for other neuropsychiatric disorders, such as schizophrenia.

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Introduction

Anti-NMDA receptor (NMDAR) encephalitis is a newly discovered autoimmune encephalitis with autoantibodies that target neuronal surface or synaptic antigens.[1] Patients present with a neuropsychiatric syndrome that typically includes acute psychosis, behavioural changes, and memory deficits, frequently followed by movement disorders (usually dyskinesia), seizures, autonomic instability, and decreased consciousness. Despite this severe clinical course, routine brain MRI is normal in most patients and therefore provides only limited diagnostic and prognostic value.[2] Anti-NMDAR encephalitis is therefore diagnosed on the basis of antibody detection in the patient's cerebrospinal fluid. By use of advanced imaging analyses, characteristic structural and functional imaging patterns of autoimmune encephalitides are increasingly being recognised.[3] In patients with anti-NMDAR encephalitis, impaired functional connectivity of the hippocampus with the medial prefrontal cortex and atrophy of the hippocampus and selected subfields has been observed and correlated with individual memory deficits.[4,5] These observations are in line with the central role of the NMDAR in learning and memory and with evidence that the hippocampus contains the highest density of NMDARs in the brain.[6] Nevertheless, NMDARs are ubiquitously expressed throughout the human brain and clinical manifestation of anti-NMDAR encephalitis clearly exceeds hippocampal dysfunction, reflecting global impairment of NMDAR function in the disease. The exact pattern of affected cortical and subcortical NMDARs, and its relation to the clinical manifestation of anti-NMDAR encephalitis, are still poorly understood. We hypothesised that clinical symptoms in the course of the disease are related to NMDAR dysfunction in functionally defined brain networks, and therefore we

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Research in context

Evidence before this study

Anti-NMDA receptor (NMDAR) encephalitis is characterised by a clinical picture that includes memory impairment, movement disorders, and psychotic symptoms, despite NMDARs being found throughout the human brain. In spite of these prominent clinical symptoms, structural MRI in patients with anti-NMDAR encephalitis is mostly normal. On June 1, 2017, we searched PubMed with the phrase "anti NMDA receptor encephalitis functional MRI" and "anti NMDA receptor encephalitis fMRI", with no date or language restrictions. This search yielded four studies that used functional MRI in participants, one of which involved 24 participants that were also in this study. The results of these studies showed decreased functional connectivity between the hippocampus and the default-mode network in correlation with memory performance, increased motor network connectivity after transcranial magnetic stimulation, increased default-mode connectivity before plasma-exchange immunotherapy in two participants, and whole-brain functional-MRI (fMRI) signal reduction in the acute disease stage that was resolved later in a single participant.

Added value of this study

This study is the first to our knowledge to investigate resting-state fMRI in a network-based, data-driven, whole-brain approach in a cohort of patients with anti-NMDAR encephalitis. We show that antibody-mediated NMDAR dysfunction results in disrupted connectivity within and between several large-scale brain networks. We also show that functional connectivity impairments in different brain regions are associated with distinct neurological and psychiatric symptoms.

Implications of all available evidence

This study helps to elucidate the pathophysiological underpinnings of anti-NMDAR encephalitis and provides an explanation as to how disruption of NMDAR function in different parts of the brain induces distinct neuropsychiatric symptoms. Finally, with anti-NMDAR encephalitis providing a unique human model of selective NMDAR disruption, our findings have implications for understanding other neuropsychiatric disorders with NMDAR dysfunction, such as schizophrenia.

investigated alterations of functional connectivity in a cohort of patients with anti-NMDAR encephalitis.

See Online for appendix

Methods

Participants

We recruited patients with anti-NMDAR encephalitis from the Department of Neurology of Charité–Universitätsmedizin Berlin. Resting-state functional MRI (fMRI) analyses of 24 of these participants have previously been reported.[4] The control group comprised age-matched and sex-matched healthy participants who did not have a history of neurological or psychiatric disorders, recruited from the general population of Berlin. Patients were studied after the acute stage of the disease and were diagnosed on the basis of characteristic clinical presentation and detection of IgG NMDAR antibodies in the cerebrospinal fluid. Two board-certified neurologists with training in psychiatry and one board-certified psychiatrist independently assessed each patient's disease severity based on the modified Rankin scale (mRS), and evaluated their neurological and psychiatric symptoms. Hallucinations, delusions, and thought disorders were classified as positive schizophrenia-like symptoms and anhedonia, avolition, flat affect, mutism, and catatonia as negative symptoms (on a binary present or absent scale, for positive and negative symptoms separately). Verbal episodic memory (Rey Auditory Verbal Learning Test [RAVLT], delayed recall) was assessed in all participants at the time of MRI. All participants gave informed written consent, and the study was approved by the Charité–Universitätsmedizin Berlin ethics committee and adheres to the STROBE statement.

MRI acquisition and fMRI preprocessing

Details are provided in the appendix. These MRI sequences were acquired on a Siemens 3T scanner (Siemens, Erlangen, Germany): T1-weighted MPRAGE sequence (1 mm isotropic); resting-state fMRI sequence (repetition time=2250 ms, voxel size=3·4 mm isotropic, 260 volumes); T2-weighted sequence; and T2-weighted FLAIR sequence. Additionally, we reviewed structural MRIs and MRI reports acquired during the acute disease stage. We did preprocessing as previously described,[7] and included rigorous control for motion artifacts, including multiple regression of 24 motion parameters, regressors for motion spikes (so-called scrubbing), and regressors for global mean, white-matter, and cerebrospinal fluid signals.

Extraction of region-wise resting-state time series

To measure functional connectivity, we first defined a whole-brain network using the Automated Anatomical Labeling (AAL) atlas, which defines 45 brain regions in each cerebral hemisphere.[8] We used intensity-based masking to avoid including voxels that are affected by signal dropout.[9] We removed regions containing fewer than 10 voxels after masking (ie, globus pallidus in all participants, olfactory cortex in five participants, gyrus rectus in one participant) from further analyses and ignored them when averaging within networks and across participants. We averaged the BOLD signal across each brain region (from grey-matter voxels only, defined using statistical parametric mapping [SPM], version 8; segmentation probability >0·5), resulting in a time series of the average activity within that region. We next computed the Pearson correlation coefficient between each pair of functional regions, resulting in a

90×90 whole-brain functional connectivity matrix. We normalised the correlation values using Fisher's *r*-to-*z* transformation.

Identification of disturbed connectivity patterns

To identify disturbed connectivity patterns across the brain, we used four approaches: voxel-wise hippocampal seed-based connectivity analysis, average connectivity in large-scale networks, direct comparison of all brain connectivity values, and logistic-regression-based feature selection.

To measure voxel-wise hippocampal connectivity, we averaged the signal from all hippocampal voxels (defined from the AAL atlas, masked with grey matter) for each participant. For each grey-matter voxel in the rest of the brain, we compared its connectivity (correlation) with the hippocampus between patients and controls using one-tailed two-sample *t* tests (false-discovery-rate [FDR] controlled for multiple comparisons).

To identify major resting-state brain networks, we averaged the full connectivity matrices of all controls to obtain a single 90×90 matrix, and applied K-means clustering on the matrix rows. The clustering approach enables identification of non-overlapping functional networks that span the whole brain, in a data-driven manner.[10,11] We did clustering with the K-means function (K=10, Euclidean distance metric) of MATLAB (version 2012a) and repeated it 100 times to avoid local minima. The number of clusters (K=10) was selected after visual inspection of results of different numbers, since the resulting clusters closely correspond to known cortical and subcortical functional networks from the literature (appendix).[10] We computed each participant's average connectivity within each network by averaging all pairwise correlation values of regions belonging to the network. We calculated inter-network connectivity as the average of all correlation values between pairs in different networks. We assessed averaged network connectivity differences between patients and controls using one-tailed two-sample *t* tests (unequal variance).

To find specific disturbed connections between brain regions, we compared the connectivity between each pair of regions between patients and controls using two-tailed two-sample *t* tests. We applied the FDR multiple-comparisons correction to the p values to identify significantly disturbed connections.

To identify the features (brain connections) that contributed the most for classification between patients and controls, we used a logistic regression classifier with L1-regularisation (λ=15, one iteration), which provides a sparse solution for feature selection, as implemented in the LIBSVM package (version 3.20).[12] Features for classification included correlation values between all brain regions (4005 values, *z* normalised). The data were classified labelled as patient or control, with leave-one-out cross validation (in each cycle, 85 participants were used for training and testing was done on the remaining participant). We did feature selection inside each cross-validation cycle on the training set by choosing all non-zero elements of the regression weights matrix. We selected discriminating features as features chosen in over two-thirds of the cross-validation cycles.

To test for correlations with clinical symptoms, for each participant we computed the within-network connectivity and the average correlation between the hippocampus and all identified voxels in the hippocampal connectivity disturbance analysis, as described previously. We then computed the correlation between these values and patients' RAVLT score, mRS score, disease duration, time after disease onset, average patient motion, number of motion spikes, and presence of psychiatric symptoms.

We applied machine learning to assess the future potential of resting-state fMRI for the classification of anti-NMDAR encephalitis from controls (appendix).

We visualised networks with Caret software (version 5.65).[13] We visualised connectivity with BrainNet Viewer (version 1.53).[14]

Data sharing

Patients' whole-brain correlation matrices and our full analysis codes are available online.

For the **data** see http://mind.huji.ac.il/nmdare.aspx

Role of the funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

We recruited 43 patients (39 women, four men; mean age 26·6 years [SD 8·6]; appendix) and 43 age-matched and sex-matched healthy participants (39 women, four men; mean age 26·9 years [SD 8·5]; p=0·9). Patients were studied after the acute stage of the disease (12·6 months [SD 2·6] after hospital discharge). In a voxel-wise analysis of hippocampal connectivity to the rest of the brain, we observed significantly reduced functional connectivity in patients with anti-NMDAR encephalitis between the two hippocampi and the medial prefrontal cortex (FDR-corrected q<0·05; figure 1; appendix). Uncorrected results showed additional reductions in connectivity to the precuneus, posterior cingulate cortex, inferior-parietal lobule, and lateral-temporal cortex—ie, the default-mode network (DMN; all uncorrected p values <0·05; figure 1; appendix).[10,15] We observed significantly reduced hippocampal connectivity independently, both in the previously investigated 24 patients,[4] as well as in the 19 newly recruited patients (p<0·0001 for both patient groups), therefore replicating and expanding on our previous results.

We investigated whether anti-NMDAR encephalitis preferentially affects distinct neuronal circuits by looking

at functional connectivity within ten major large-scale resting-state brain networks,[10] and compared network connectivity values between severely affected patients (mRS >2), moderately affected patients (mRS ≤2), and controls (appendix). For moderately affected patients, there was a significant reduction in connectivity within the medial-temporal lobe (MTL) network (mean r=0·34 [controls], 0·28 [patients]; difference 95% CI 0·04–0·08; FDR-corrected p=0·0005) and in the sensorimotor network (mean r=0·53 [controls], 0·44 [patients]; 0·05–0·13; FDR-corrected p=0·01 figure 2). In severely affected patients, we identified additional connectivity reductions in the visual network (mean r=0·53 [controls], 0·46 [patients], difference 95% CI 0·02–0·12; FDR-corrected p=0·043) and lateral-temporal network (mean r=0·24 [controls], 0·18 [patients]; 0·03–0·09; FDR-corrected p=0·028; figure 2). There was no significant reduction in connectivity in any other of the identified large-scale brain networks (DMN, ventral attention network, auditory network, thalamus-basal ganglia network, frontoparietal control network, and orbitofrontal-temporal network). We also assessed connectivity between each network and the other nine networks. This analysis showed a significant reduction in connectivity only between the MTL network and DMN, providing support to the hippocampal seed-based analysis, although this result was not significant after correction for multiple comparisons (mean r=0·10 [controls], 0·07 [patients]; difference 95% CI 0·01–0·06, uncorrected p=0·011). All of the identified network connectivity reductions were also significant when comparing only the 39 female patients to the 39 female controls (FDR-corrected p <0·05).

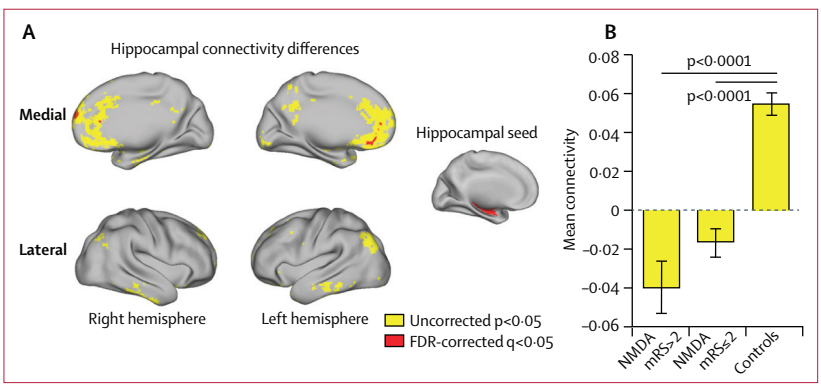

Figure 1: **Connectivity changes between the hippocampus and cerebral cortex in patients with anti-NMDA receptor encephalitis**
(A) Voxels that showed significantly decreased connectivity to the hippocampus in patients with anti-NMDA receptor encephalitis are shown in red (FDR-corrected results; q<0·05) and yellow (uncorrected results; p<0·05). The pattern of decreased connectivity clearly delineates the default-mode network (appendix). The hippocampal seed region of interest is shown for visual reference. (B) Functional connectivity between the hippocampus and the voxels (identified in panel A), shown separately for severely affected patients (mRS >2), moderately affected patients (mRS ≤2), and controls. Black lines indicate significant differences (p<0·0001). Error bars show SEM values. FDR=false-discovery rate. mRS=modified Rankin scale.

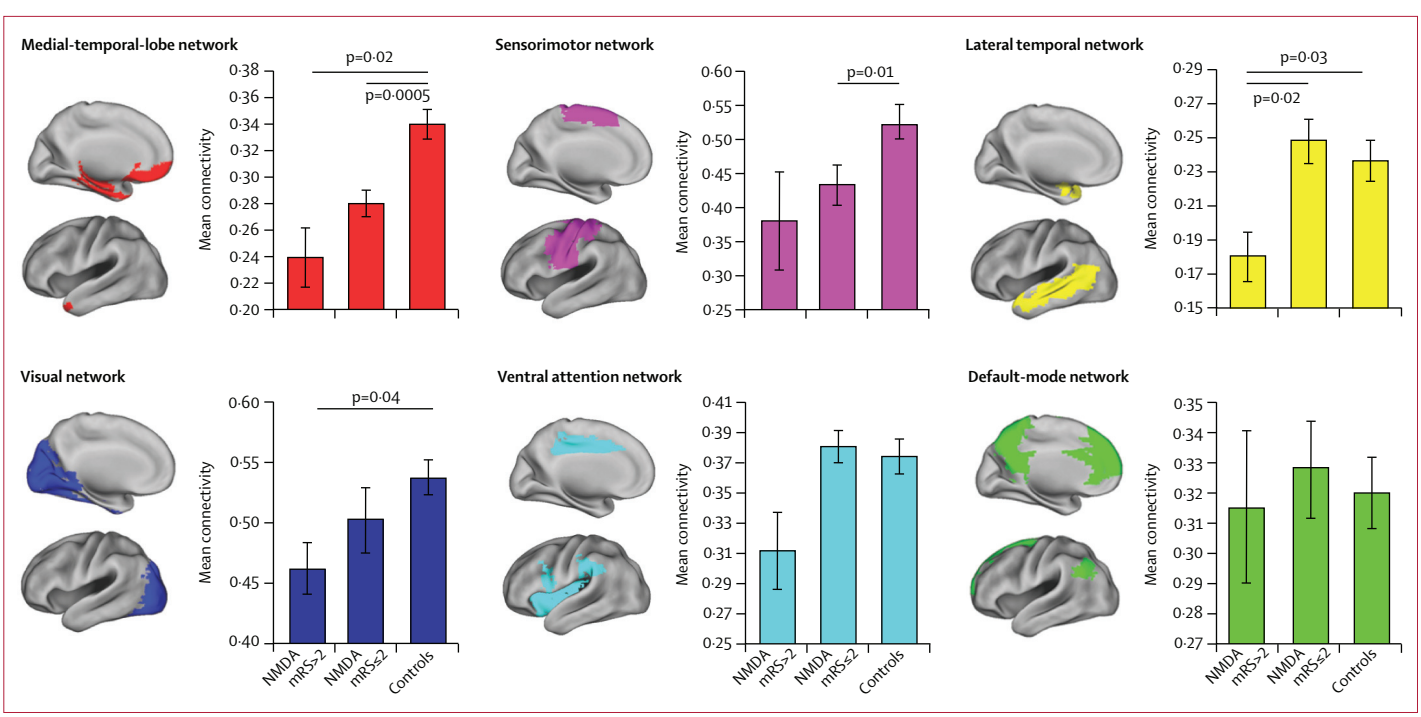

Figure 2: **Functional connectivity reductions in major brain networks in patients with anti-NMDAR encephalitis**
We identified ten functional networks by clustering connectivity patterns in controls. Four networks (medial-temporal, sensorimotor, lateral-temporal and visual) showed significant changes, and a similar but not significant trend was observed for the ventral attention network, while no connectivity changes were observed within the default-mode network. Connectivity reduction is shown separately for severely affected patients (mRS >2), moderately affected patients (mRS ≤2), and controls. Bar graph values indicate mean within-network connectivity, computed as mean connectivity between all pairs of regions belonging to the network. Black lines indicate a significant decrease in connectivity between patients and controls (FDR-corrected p<0·05). All networks are projected on the left hemisphere.

We directly compared the connectivity values between patients and controls for all pairs of brain regions (as identified in the AAL atlas[8]), to identify specifically disrupted connections. We observed significantly reduced functional connectivity for 16 pair-wise connections (FDR-corrected p<0·05). All of these connections included frontal-lobe regions and 75% of these had reduced connectivity with medial-temporal and inferior-parietal regions (figure 3).

To validate these results using a complementary approach, we used logistic regression classification, which identifies strongly discriminating features (brain connections) between patients and controls. 29 brain connections were identified as important for classification (appendix). These connections were mostly between medial-frontal and medial-temporal regions (16 connections), between lateral-frontal and lateral-parietal regions (six connections), or between sensory-motor regions (three connections), corroborating the results of the previous analyses. Using only these brain connections, the classifier separated patients from controls (69% correct prediction; permutation test p=0·025). Additionally, a supervised machine-learning analysis discriminated with high accuracy (81%) between patients and controls on the basis of functional connectivity patterns (appendix).

To examine the clinical relevance of the identified functional connectivity decreases, we correlated patients' clinical parameters with hippocampal connectivity impairment and connectivity within large-scale networks (appendix). Hippocampal connectivity correlated significantly with RAVLT delayed recall performance in patients (r=0·33, β=24·1; p=0·031). Furthermore, connectivity within the MTL network was significantly correlated with memory performance in the RAVLT delayed recall score (r=0·35, β=18·9; p=0·021). RAVLT scores ranging across the full possible spectrum (1–15) and high regression slopes (β values) support the clinical relevance of the relation between connectivity changes and memory performance (appendix). Alteration of functional connectivity in the affected networks (MTL, sensorimotor, lateral-temporal, and visual) scaled with disease severity, because connectivity in severely affected patients was lower than in moderately affected patients (figure 2). Finally, positive schizophrenia-like symptoms were correlated with connectivity within the frontoparietal control network (r=0·34, β=2·48; p=0·026) and inversely correlated with connectivity within the lateral-temporal network (r=–0·30, β=–1·63; p=0·050) whereas negative psychotic symptoms were correlated with connectivity within the ventral attention network (r=0·32, β=2·33; p=0·036). Among strongly discriminating brain connections, average frontoparietal connectivity was correlated with positive psychotic symptoms (r=0·31, β=1·68; p=0·047), corroborating the average network correlation findings. The described correlations were not corrected for multiple comparisons, because of the small number of patients and the exploratory nature of this analysis. There was no significant correlation of functional connectivity with disease duration, time since disease onset, patient age, or patient's mean head motion. Further demonstrations that motion did not affect the observed connectivity reductions are provided in the appendix.

Analysis of structural images (T1, T2, and FLAIR-weighted) acquired during the acute stage of the disease did not detect any significant abnormality in 31 (72%) of 43 patients (appendix).

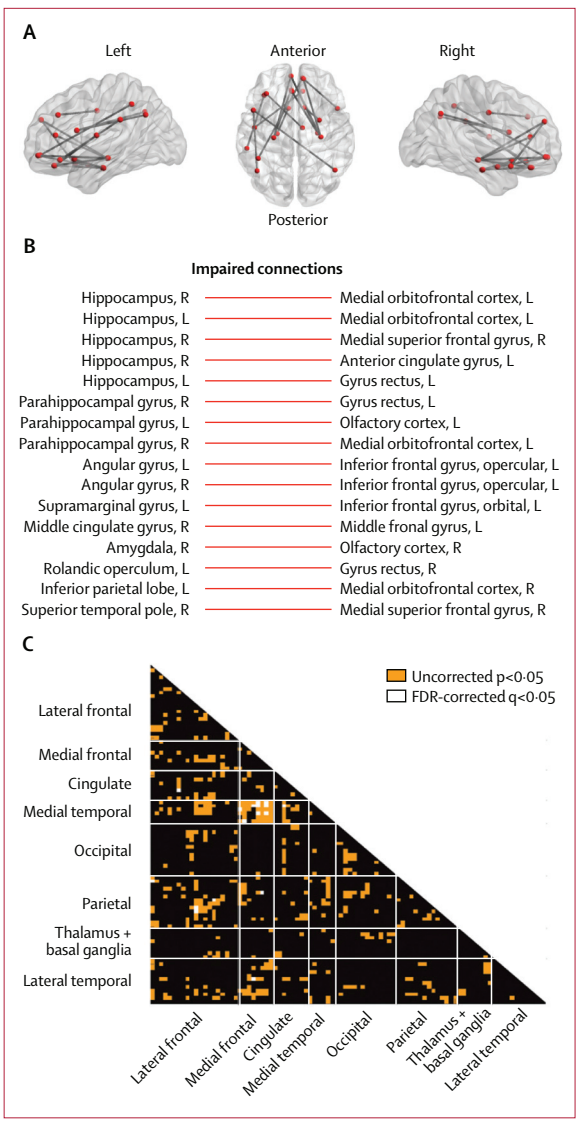

Figure 3: **Specific brain connections with highly decreased connectivity in patients with anti-NMDAR encephalitis**
(A) Decreased connectivity between brain regions is marked on a glass brain in lateral and dorsal views. (B) Regions involved in each affected connection. Affected connections involve mainly the frontal and medial temporal lobes. (C) Significant connectivity differences between patients and controls for all brain regions (in the order of the Automated Anatomical Labeling atlas).

Discussion

Network analysis of resting-state fMRI data recorded from patients with anti-NMDAR encephalitis revealed characteristic patterns of alterations in whole-brain functional connectivity. There were inter-network perturbations between the hippocampus and MTL and the DMN, and significant reductions in intra-network connectivity were observed within the MTL, sensorimotor, lateral-temporal, and visual networks, when compared with controls. Logistic regression classification corroborated the findings of fronto-parietal and frontotemporal connectivity disturbances. Exploratory analyses indicated that impaired fronto-temporal connectivity and decoupling of the MTL network and the DMN are associated with impaired memory performance. Additionally, in line with the proposed NMDAR dysfunction in schizophrenia,[16–18] we observed associations between positive and negative schizophrenia-like symptoms and alteration in functional connectivity of the frontoparietal control network and the ventral attention network. Finally, supervised machine-learning analyses discriminated, with high accuracy, between patients and controls on the basis of functional connectivity patterns, providing a preliminary demonstration of the potential clinical use of resting-state fMRI—by contrast with structural routine MRI—to contribute meaningful information on the disease state. This potential use requires validation in longitudinal studies and comparison with patient groups with similar clinical presentation.

The hippocampus plays a central role in the pathophysiology of anti-NMDAR encephalitis, as reflected by severe and persistent memory deficits and structural and functional MRI findings.[4,5,19,20] In this study, we observed impaired functional connectivity of the hippocampus using four complementary approaches: reduced functional connectivity within the MTL network, reduced seed-based functional connectivity between the hippocampus and major nodes of the DMN (predominantly the medial prefrontal region), decoupling of the MTL and DMN networks, and disturbed frontotemporal connectivity in the whole-brain pair-wise connectivity analysis and logistic regression analysis. These results complement previous observations of reduced hippocampal functional connectivity with the anterior DMN.[4] Reduced connectivity of the hippocampus and within the MTL network was associated with impaired memory. These findings emphasise the pathophysiological and clinical relevance of impaired hippocampal NMDAR function for memory deficits in these patients, and provide a model for the role of NMDARs in human memory.[4,5,19]

We observed no alterations in connectivity within the DMN, although the hippocampus is frequently considered as part of this network. This contrasts with a previous report of DMN disturbances in a patient in the acute encephalitis stage.[20] The DMN is involved in internally directed processes, including mind-wandering, theory of mind, autobiographical memory, and mental orientation.[15,21] Coupling of the hippocampus with the DMN is flexible and can depend on the behavioural context, with DMN-hippocampus dissociation during memory encoding.[22] Our results show that anti-NMDAR encephalitis causes a disruption of the coupling between the hippocampus and the DMN, rather than disturbing connectivity within the DMN. Intriguingly, similar patterns of disrupted MTL–DMN connectivity have been observed in other disorders with prominent memory deficits, such as Alzheimer's disease,[23] and after administration of the NMDAR antagonist ketamine.[24]

Beyond the hippocampus, functional connectivity disturbances were widespread and affected distributed large-scale brain networks—ie, the MTL, sensorimotor, visual, and lateral-temporal networks. In line with these observations, a case study of a patient with NMDAR encephalitis found functional connectivity changes in several large-scale resting-state networks in the acute disease stages.[20] Together, these findings reflect the expression of NMDARs throughout the cortex and highlight their central role in behaviour.[6] The widespread connectivity disturbances could also be understood in light of the previously described white-matter damage in anti-NMDAR encephalitis, which might cause functional connectivity disturbances without apparent grey-matter damage.[4,25]

Our findings suggest that functional connectivity impairments represent biologically plausible correlates of major disease symptoms that are in line with clinical knowledge and theories, although these results require further testing because they did not pass multiple comparisons correction. Psychotic symptoms were correlated with frontoparietal connectivity disturbances (in the ventral attention and frontoparietal control networks and individual brain connections), in accordance with recent findings of ventral attention network and fronto-parietal control network disturbances in schizophrenia,[24] and with models of psychosis that suggest reliance on NMDAR regulation and frontoparietal connectivity.[16] The large regression coefficients in our study suggest clinical relevance of the observed connectivity changes for psychotic symptoms. Disturbance of sensorimotor connectivity might be related to movement disorders in acute disease stages, in accordance with ^{18}F-fluorodeoxyglucose (^{18}F-FDG) PET and transcranial magnetic stimulation findings of motor cortex dysfunction in patients with anti-NMDAR encephalitis.[26,27] The results of visual network disturbances are in line with recent findings of reduced visual acuity in these patients that correlated with disease severity,[28] suggesting a cortical origin for these deficits and corroborating findings of reduced occipital ^{18}F-FDG-PET metabolism in the disease.[29] Finally, disruption of executive function in patients with anti-NMDAR encephalitis[19] could be related to the abnormal frontal functional connectivity we have observed.

Despite our systematic findings, this study is not free of limitations. First, our patients were scanned after the acute disease stage and connectivity disturbances thus reflect the long-term effects of the disorder. The use of clustering and averaging activity across functional clusters assumes homogeneous activity within them; negative correlations inside functional clusters could complicate interpretation, and alternative measures such as principal eigenvariates could be considered in future. However, this consideration does not affect results regarding the location of connectivity changes in patients. Additionally, results of connectivity correlation to clinical parameters are exploratory because they did not pass multiple comparisons correction, and they therefore need to be validated in large patient groups. Although we used stringent motion correction and confounds regression, alternative analyses, such as physiophysiological interactions, that further eliminate noise from the data might be of interest in the future. Finally, individually adjusted parcellations could provide even more precise results than the AAL parcellation we applied.

We have shown that anti-NMDAR encephalitis is associated with connectivity disturbances predominantly between frontotemporal cortices, but also in sensorimotor, visual, frontoparietal, and MTL networks. This dysfunction is associated with major disease symptoms, highlighting the role of NMDAR distribution and associated network function in psychosis and memory. Analysis of functional connectivity thus provides a valuable tool to advance the pathophysiological understanding of anti-NMDAR encephalitis and other disorders related to NMDAR dysfunction.

Contributors

MP, FP, SA, and CF designed research. HP and CF collected data. MP, IB-D, SA, and CF analysed data. MP, HP, FP, SA, and CF wrote the paper.

Declaration of interests

We declare no competing interests.

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References

1 Graus F, Titulaer MJ, Balu R, et al. A clinical approach to diagnosis of autoimmune encephalitis. *Lancet Neurol* 2016; **15:** 391–404.

2 Dalmau J, Lancaster E, Martinez-Hernandez E, Rosenfeld MR, Balice-Gordon R. Clinical experience and laboratory investigations in patients with anti-NMDAR encephalitis. *Lancet Neurol* 2011; **10:** 63–74.

3 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.

4 Finke C, Kopp UA, Scheel M, et al. Functional and structural brain changes in anti-N-methyl-D-aspartate receptor encephalitis. *Ann Neurol* 2013; **74:** 284–96.

5 Finke C, Kopp UA, Pajkert A, et al. Structural hippocampal damage following anti-N-methyl-D-aspartate receptor encephalitis. *Biol Psychiatry* 2016; **79:** 727–34.

6 Palomero-Gallagher N, Amunts K, Zilles K. Transmitter receptor distribution in the human brain. In: Toga AW, ed. Brain Mapping. Amsterdam: Elsevier, 2015: 261–75.

7 Peer M, Nitzan M, Goldberg I, et al. Reversible functional connectivity disturbances during transient global amnesia. *Ann Neurol* 2014; **75:** 634–43.

8 Tzourio-Mazoyer N, Landeau B, Papathanassiou D, et al. Automated anatomical labeling of activations in SPM using a macroscopic anatomical parcellation of the MNI MRI single-subject brain. *Neuroimage* 2002; **15:** 273–89.

9 Peer M, Abboud S, Hertz U, Amedi A, Arzy S. Intensity-based masking: a tool to improve functional connectivity results of resting-state fMRI. *Hum Brain Mapp* 2016; **37:** 2407–18.

10 Yeo BTT, Krienen FM, Sepulcre J, et al. The organization of the human cerebral cortex estimated by intrinsic functional connectivity. *J Neurophysiol* 2011; **106:** 1125–65.

11 Craddock RC, James GA, Holtzheimer PE, Hu XP, Mayberg HS. A whole brain fMRI atlas generated via spatially constrained spectral clustering. *Hum Brain Mapp* 2012; **33:** 1914–28.

12 Chang C-C, Lin C-J. LIBSVM: a library for support vector machines. *ACM Trans Intell Syst Technol* 2011; **2:** 1–27.

13 Van Essen DC, Drury HA, Dickson J, Harwell J, Hanlon D, Anderson CH. An integrated software suite for surface-based analyses of cerebral cortex. *J Am Med Informatics Assoc* 2001; **8:** 443–59.

14 Xia M, Wang J, He Y. BrainNet Viewer: a network visualization tool for human brain connectomics. *PLoS One* 2013; **8:** e68910.

15 Raichle ME. The brain's default mode network. *Annu Rev Neurosci* 2015; **38:** 433–47.

16 Stephan KE, Friston KJ, Frith CD. Dysconnection in schizophrenia: from abnormal synaptic plasticity to failures of self-monitoring. *Schizophr Bull* 2009; **35:** 509–27.

17 Krystal JH, D'Souza DC, Mathalon D, Perry E, Belger A, Hoffman R. NMDA receptor antagonist effects, cortical glutamatergic function, and schizophrenia: toward a paradigm shift in medication development. *Psychopharmacology (Berl)* 2003; **169:** 215–33.

18 Moghaddam B, Javitt D. From revolution to evolution: the glutamate hypothesis of schizophrenia and its implication for treatment. *Neuropsychopharmacology* 2012; **37:** 4–15.

19 Finke C, Kopp UA, Prüss H, Dalmau J, Wandinger K-P, Ploner CJ. Cognitive deficits following anti-NMDA receptor encephalitis. *J Neurol Neurosurg Psychiatry* 2012; **83:** 195–98.

20 Brier MR, Day GS, Snyder AZ, Tanenbaum AB, Ances BM. N-methyl-D-aspartate receptor encephalitis mediates loss of intrinsic activity measured by functional MRI. *J Neurol* 2016; **263:** 1083–91.

21 Peer M, Salomon R, Goldberg I, Blanke O, Arzy S. Brain system for mental orientation in space, time, and person. *Proc Natl Acad Sci USA* 2015; **112:** 11072–77.

22 Huijbers W, Pennartz CMA, Cabeza R, Daselaar SM. The hippocampus is coupled with the default network during memory retrieval but not during memory encoding. *PLoS One* 2011; **6:** e17463.

23 Celone KA, Calhoun VD, Dickerson BC, et al. Alterations in memory networks in mild cognitive impairment and Alzheimer's disease: an independent component analysis. *J Neurosci* 2006; **26:** 10222–31.

24 Anticevic A, Gancsos M, Murray JD, et al. NMDA receptor function in large-scale anticorrelated neural systems with implications for cognition and schizophrenia. *Proc Natl Acad Sci USA* 2012; **109:** 16720–25.

25 Hacohen Y, Absoud M, Hemingway C, et al. NMDA receptor antibodies associated with distinct white matter syndromes. *Neurol Neuroinflammation* 2014; **1:** e2.

26 Maeder-Ingvar M, Prior JO, Irani SR, Rey V, Vincent A, Rossetti AO. FDG-PET hyperactivity in basal ganglia correlating with clinical course in anti-NDMA-R antibodies encephalitis. *J Neurol Neurosurg Psychiatry* 2011; **82:** 235–36.

27 Volz MS, Finke C, Harms L, et al. Altered paired associative stimulation-induced plasticity in NMDAR encephalitis. *Ann Clin Transl Neurol* 2016; **3:** 101–13.

28 Brandt AU, Oberwahrenbrock T, Mikolajczak J, et al. Visual dysfunction, but not retinal thinning, following anti-NMDA receptor encephalitis. *Neurol Neuroimmunol Neuroinflammation* 2016; **3:** e198.

29 Leypoldt F, Buchert R, Kleiter I, et al. Fluorodeoxyglucose positron emission tomography in anti-N-methyl-D-aspartate receptor encephalitis: distinct pattern of disease. *J Neurol Neurosurg Psychiatry* 2012; **83:** 681–86.