Neuronal morphology and physiology, functional brain networks and memory in temporal lobe epilepsy

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ABSTRACT

Temporal lobe epilepsy patients are heterogeneous regarding cognitive functioning, with predominant risk of memory deficits. Despite major advances within cellular neuroscience, neuroimaging, and neuropsychology, it remains challenging to integrate memory performance with cellular characteristics and brain network topology. In a unique dataset, we investigate these cross-scale individual differences. Preoperatively, drug-resistant temporal lobe epilepsy patients (*n* = 31, 15 females) underwent functional magnetic resonance imaging, magnetoencephalography and/or memory testing. Macro-scale network centrality was determined, since the number of integrative functional connections a region has is crucial for memory functioning. Subsequently, non-pathological cortical tissue resected from the lateral middle temporal gyrus (default mode network) was used for single cell morphological (total dendritic length) and electrophysiological patch-clamp analysis (action potential rise speed). We expected greater macro-scale centrality to relate to longer micro-scale dendritic length and faster

action potentials, and greater centrality to relate to better memory performance. Greater macro-scale centrality correlated with longer dendritic length and faster action potentials (canonical correlation coefficient = 0.329, $p < 0.001$). Moreover, greater macro-scale centrality was related to better memory performance (canonical correlation coefficient = 0.234, $p = 0.013$). We conclude that more complex neuronal morphology and faster action potential kinetics are mirrored by more integrative functional network topology of the middle temporal gyrus, which in turn is associated with better memory functioning. Thus, our cross-scale analyses reveal a significant relationship between cellular and imaging measures of network topology in the brain, which support cognitive performance in these patients.

Introduction

A long-standing question in clinical neuroscience is how to integrate vastly different scales of investigation to better understand the brain. Cross-scale analysis of neural organizational principles in the same individual is typically difficult: mechanistic work is performed at the level of individual neurons in animals or postmortem samples, while clinical research takes place at the level of behavioral testing and macro-scale neuroimaging, leaving a wide translational gap between the two. In patients with temporal lobe epilepsy (TLE), both scales of investigation have been exploited: a subgroup of patients undergoes craniotomy to resect the presumed seizure origin, allowing for histopathological work that has given major insights into disease pathophysiology [1]. Patients also undergo an extensive clinical work-up before surgery, including imaging, neurophysiological recording and cognitive testing [2,3]. However, an understanding of often-reported and clinically relevant individual differences across scales is lacking.

Recent macro-scale investigations suggest that cognition may be explained by whole-brain structural connections between brain areas, and the orchestrated functional dynamics taking place on top of that structural network [4,5]. Network neuroscience allows for characterization of this network, consisting of nodes (brain regions) and edges between them (structural or functional connections). While structural connections are usually determined using diffusion magnetic resonance imaging (dMRI), functional connections can be estimated using resting-state functional MRI (rsfMRI) or magnetoencephalography (MEG), assessing different aspects of synchronized brain activity. Despite using different modalities, functional network topology has consistently been shown to be relevant for cognitive capabilities in healthy persons [6], in patients suffering from neurological disease [4] and in TLE patients specifically [7,8]. An open question is

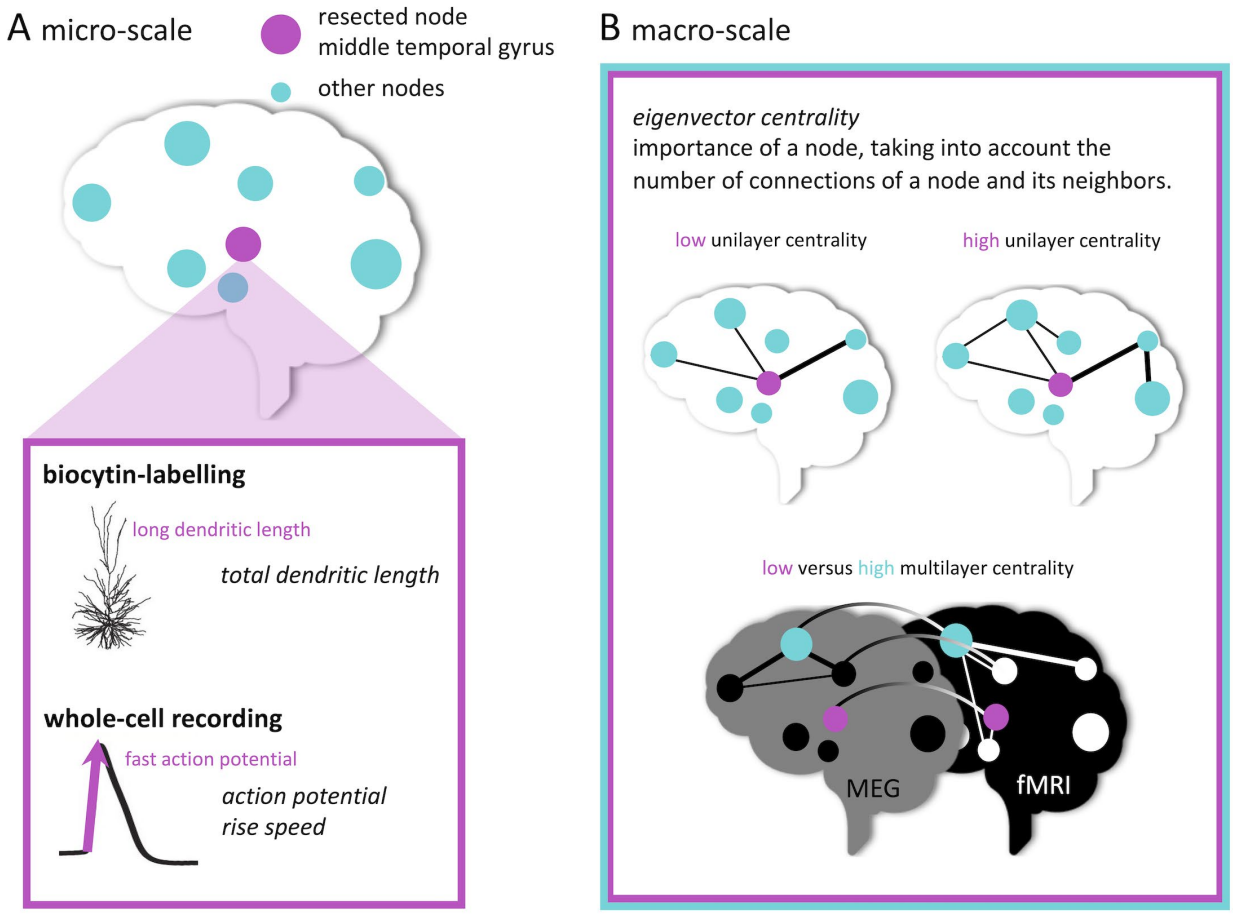
whether combining different imaging and neurophysiological modalities may improve explanation of cognitive variance in these patients. Multilayer network theory provides a framework to integrate such modalities into a single network consisting of interconnected layers [9]. Previous work suggests that multilayer functional brain network properties supersede unilayer network characteristics in explaining cognitive functioning in Alzheimer's disease patients [10].

The most important properties of brain networks in relation to cognition are segregation and integration [11–13]. Segregation refers to the extent to which nodes are locally interconnected, and integration signifies the level of integrative connectivity taking place, either in globally or at a particular node. At specific brain regions, the extent of segregation and integration typically show opposite patterns [14,15]: certain regions have a more segregative topological role (e.g. regions within the visual system), while others are considered mainly integrative (e.g. regions in the frontal lobe), indicating that brain regions tend to have a 'typical' role in the brain network that can be quantified with either segregative or integrative network measures. For example, centrality indicates the expected level of network integration occurring at any particular node (see figure 1B on the next page), with nodes showing high integration most likely having low segregation.

Cognitively impaired TLE patients, with pathology of and around the hippocampus, predominantly suffer from memory dysfunction [16]. Integrative connectivity of the temporal lobe, also overlapping with the default mode network (DMN [17]), may be paramount to explain individual memory differences [18–20]. An exemplar study explored the integrative connectivity

of the hippocampal circuit with the posterior DMN [21], reporting that greater memory deficits were related to lower network integration.

Figure 1. Schematic representation of multi-scale analyses



Legend. In (A), micro-scale tissue collection from the middle temporal gyrus (pink node) is depicted. Morphological analysis and electrophysiological recordings were performed. In (B), the macro-scale network measure of eigenvector centrality is illustrated for the unilayer (fMRI or MEG) and multilayer (combined fMRI and MEG) network analyses.

It has been considerably more difficult to assess micro-scale brain properties in relation to human cognitive variance. Memory disturbances in TLE have been related to micro-scale

properties of the (medial) temporal lobe in terms of protein expression [22]. Recently, morphological and physiological properties of individual neurons were shown to explain variance in intelligence in TLE patients [23]. Pyramidal neurons in resected non-pathological tissue from the non-pathological temporal cortex of patients with higher intelligence had larger, more complex dendrites and faster action potentials during high frequency activity. Larger dendrites may enable neurons to have more synaptic contacts, putatively playing more integrative roles than neurons with smaller dendrites [24,25]. Larger dendrites also directly influence the speed of action potential initiation, possibly providing these cells with better temporal resolution and more efficient information transfer [23,25,26]. Thus, regions that act as integrators for cognitive processes may be characterized by neurons with larger dendrites and faster action potentials.

Others have also started to hypothesize on possible links between micro-scale brain properties and macro-scale network profiles [27,28], with a recent review on "microstructure-informed connectomics" further underlining the potential for cross-linking between different scales in order to increase our understanding of the brain and treatment of disease [29]. These efforts are all the more relevant as large-scale initiatives like the Human Brain Project strive towards integration of our knowledge between these different scales of measurement. Indeed, studies have shown that regional variations in integration across scales go hand in hand: more integrative macro-scale brain regions as measured with diffusion weighted imaging tend to have bigger neurons with more axons than less integrative regions, as shown in animal and postmortem work [30,31]. Translation of these cross-scale associations to living humans, and their cognitive correlates, remain unknown.

Therefore, we investigated a unique cohort of TLE patients characterized at multiple scales. First, patients underwent cognitive assessments, neuroimaging and neurophysiological recording (fMRI and MEG [32,33]). Then, patients underwent neurosurgery and electrophysiological, patch-clamp recordings were performed on individual pyramidal neurons from the resected non-pathological temporal cortex tissue to characterize functional properties [23,34]. In parallel, dye labeling of recorded neurons enabled post-hoc quantification of neuronal morphology at (sub)cellular resolution. This pipeline allowed us to test whether individual differences covaried across scales, such that larger dendritic length and faster action potentials of the temporal region would associate with higher functional network centrality of this node. We also explored whether the macro-scale properties of the region related to individual differences in memory functioning, expecting that greater centrality of this DMN region relates to better memory performance.

Results

Patients

All patients undergoing resective neurosurgery for drug-resistant epilepsy localized at the medial temporal lobe between 2009-2016 at Amsterdam University Medical Centers (location VUmc, Amsterdam, The Netherlands) were eligible for participation. All patients underwent temporal lobectomy and were included in a previously published study [23]. In 15 of the 46 patients originally included, MEG and fMRI were not available. Finally, 31 patients (15 females) were included (see Table 1 on the next page for detailed characteristics per patient) with a mean age of 33 years (± 11 years).

A schematic of the analysis is provided in Figure 1 on page 6. The tissue collection, as described in [23], formed the basis for further analyses (see Materials and Methods for detailed information). In short, tissue exclusively originated from the middle temporal gyrus and was removed in order to gain access to the disease focus in deeper lying structures. In all patients, the resected neocortical tissue was not part of the epileptic focus or tumor and displayed no structural/functional abnormalities in presurgical MRI investigation or histological analysis by an experienced pathologist. This tissue was then analyzed with single cell electrophysiology and morphology, yielding a number of parameters. As our three measures of integration at the micro-scale, we selected total dendritic length, rise of the 1st action potential (AP), and APs fired at frequencies between 20-40 Hz (see Materials and Methods) for further cross-scale analysis, due to their proven relevance for intelligence in this patient cohort [23].

Table 1. Patient characteristics

ID	fMRI	MEG	Ephys	Morph	Sex	Age	Lat	Dom	Onset	Dur	Freq	Etiol	Type	AED	IR	DR	WMS
1	A	NA	A	A	F	21	R	Non	11	10	32	LGG	SP	LEV | VPA	49	10	85
2	A	NA	A	A	F	31	L	Non	16	15	13	LGG	CP	CBZ | LEV	55	6	100
3	A	A	A	A	F	29	L	Dom	13	17	32	HS	CP | GTC	LTG | TPM	48	5	NA
4	NA	A	A	A	M	20	R	Non	16	5	2	DNET	CP	CBZ | LEV	52	NA	NA
5	A	NA	A	A	F	27	R	Non	14	13	120	DNET	SP	CBZ | LTG	28	7	90
6	NA	A	A	NA	M	18	R	Non	1	17	150	HS	CP	OXC	NA	NA	NA
7	A	A	NA	A	F	23	L	NA	15	8	9	HS	SP | GTC	LEV | OXC	35	1	82
8	A	A	NA	A	M	53	L	Non	6	47	10	LGG	CP	CBZ | CLB	25	9	65
9	A	NA	NA	A	M	19	R	Non	17	2	450	GLI	CP	CLB | OXC	NA	NA	75
10	NA	A	NA	A	F	35	L	NA	1	34	1	NA	SP | CP | GTC	CZP | LCS | LTG | LEV	36	9	78
11	NA	A	NA	A	M	25	L	NA	9	16	2	HS	SP | CP | GTC	CLB | LCS | LEV	52	12	NA
12	NA	A	A	A	F	31	R	Dom	21	10	4	HS	CP	CBZ | CLB	41	1	80
13	NA	A	A	A	M	49	L	Dom	8	41	2	HS	CP	CBZ | CLB | LEV	45	9	NA
14	A	NA	A	A	F	45	R	Dom	23	22	3	HS	CP	CBZ | CLB | LTG	51	11	101
15	A	NA	A	NA	M	38	R	Non	28	10	6	HS	CP	CBZ	NA	NA	110
16	A	NA	A	NA	M	44	L	NA	35	9	4	HS	SP	LCS | VPA	NA	NA	102
17	NA	A	A	NA	M	53	L	Dom	18	35	4	NA	CP	CBZ	NA	NA	NA
18	NA	A	NA	A	F	30	L	NA	2	28	9	HS	CP | GTC	CLB | OXC	54	1	70
19	NA	A	A	A	M	44	L	NA	4	40	8	HS	CP | GTC	LTG | LEV	30	10	83
20	NA	A	A	NA	F	32	R	NA	6	26	151	MCD	NA	NA	NA	NA	NA
21	A	A	A	NA	F	41	R	Non	8	33	6	GLI	AB | CP	LEV | CBZ | VPA | CLB	44	NA	86
22	A	NA	A	NA	M	29	R	Non	23	7	5	NA	AB | CP	PHB | CBZ	28	NA	NA
23	A	A	A	NA	F	20	R	Non	4	17	6	MCD	CP	LEV | CBZ	58	NA	NA
24	NA	A	A	NA	M	21	L	NA	8	13	6	GG	AB	CBZ | LEV | LTG	NA	NA	84
25	NA	A	A	NA	F	48	R	NA	14	34	48	HS	AB | GTC	ZNS | CBZ | VPA	48	NA	104
26	A	NA	A	NA	M	44	R	Non	25	19	1	HS	CP	LTG | CBZ	NA	NA	NA
27	NA	A	A	NA	M	43	R	NA	6	37	7	HS	SP | CP	OXC | LEV	NA	NA	NA
28	A	A	A	NA	F	33	R	Non	19	14	150	HS	AB | CP	CBZ | LEV | CLB	51	NA	80
29	A	A	A	NA	M	51	R	Non	4	48	60	NA	CP | GTC	CBZ | PHB	41	NA	NA
30	A	A	A	A	F	18	R	Non	5	13	17	GG	CP	OXC	66	NA	NA
31	A	A	A	A	M	23	R	Non	11	12	8	HS	CP	OXC	56	NA	83

Legend. ID = identification number, fMRI = functional magnetic resonance imaging, MEG = magnetoencephalography, Ephys = electrophysiology, Morph = morphology, Lat = lateralization of epileptic focus, Dom = dominant hemisphere of resection, Onset = age in years at seizure onset, Dur = duration of epilepsy in years, Freq = frequency of seizures per month, Etiol = etiology of epilepsy, Type = type of epilepsy, AED = anti-epileptic drugs, IR = immediate recall on the Rey Auditory Verbal Learning Test, DR = delayed recall on the Rey Auditory Verbal Learning Test, WMS = Wechsler Memory Scale Verbal Memory Index, A = available, NA = not available, F = female, M = male, R = right, L = left, Non = non-dominant, Dom = dominant, LGG = low-grade glioma, HS = hippocampal sclerosis, DNET = dysembrionic neuroepithelial tumor, CAV = cavernoma, GLI = gliosis, MCD = malformation of cortical development, GG = ganglioglioma, SP = simpel partial, CP = complex partial, GTC = generalized tonic clonic, AB = absence, LEV = levetiracetam, VPA = valproic acid, CBZ = carbamazepine, CLB = clobazam, OXC = oxcarbazepine, LTG = lamotrigine, LCS = lacosamide, PHB = phenobarbital.

For fMRI and MEG analysis, the same structural MRI was used to coregister the functional data, ensuring spatial comparability between modalities. For fMRI, we used standard and previously published pre-processing of pseudo resting-state data to extract extensively motion-corrected time series for all regions with reliable signal in an atlas spanning 78 regions of the cortex [35]. Pearson's correlations were used to construct the links between these nodes, yielding a single network per patient. For MEG, we used standard and previously published pre-processing of resting-state recordings and subsequent beamforming to extract time series from the same cortical regions [32,33]. Connections between these regions were constructing using the phase lag index [36], yielding a single network per patient. Centrality was then calculated for the region from which the tissue was taken specifically, for both the fMRI and MEG networks, as well as for a multilayer network that combined fMRI and MEG networks. Viewing different network modalities separately may decrease the explanatory power of network measures [68–70]. Multilayer network theory [71,72] offers an analytical framework that allows for synergy between modalities to be captured. Each layer in a multilayer network represents a network characterized by one type of connectivity. In a multiplex network (a subcategory of multilayer networks), interlayer connections then link the same region across different layers. Importantly, multilayer network measures supersede summed properties of individual layers when trying to explain system behavior [73,74]. Multilayer techniques have only recently been applied to neuroscience [37,38,75,76], but show promising results towards explaining more cognitive variance than unilayer analyses in Alzheimer's disease patients [10]. Ultimately, this yielded three centrality values per patient (fMRI, MEG, multilayer).

Finally, patients underwent cognitive assessments during presurgical workup, as reported before [23]. As our three measures of verbal memory, we selected (1) the composite Verbal Memory Index of the Wechsler Memory Scale (WMS [66]), and (2) immediate and (3) delayed recall of the Dutch version of Rey's Auditory Verbal Learning Test (RAVLT [67]). Higher values indicate better memory performance (see Materials and Methods for detailed information).

Sample sizes varied per scale, as indicated in Table 1. We therefore first performed multiple imputation of missing data based on patterns of covariation within the available data [82], namely (1) the three micro-scale measures per patient (TDL, AP rise speed (1st), and AP rise speed (20-40Hz)), (2) the three macro-scale centrality values per patient (MEG, fMRI and multilayer), and (3) the three memory scores per patient (WMS, RAVLT immediate and delayed recall). We thereby created ten imputed datasets, on which we performed cross-scale canonical correlation analyses to test our primary hypotheses. If significant, these canonical correlation analyses were then followed by correlational analysis on the non-imputed samples.

Associations between micro-scale and macro-scale brain properties

We first asked whether longer TDLs and faster APs, hypothetically signs of greater integrative potential at the micro-scale, generally related to greater macro-scale network centrality of the resected region, as such a correlation would indicate that integrative properties are preserved across scales. Indeed, there was a significant cross-scale correlation in the imputed data (canonical correlation coefficient = 0.329, $F(9,740) = 7.339$, $p < 0.001$, $n = 31$).

We then investigated pairwise correlations in non-imputed data (Figure 2 on page 14). Longer micro-scale TDL was significantly related to greater macro-scale fMRI centrality (rho =

0.758, CI [0.115 0.975], p = 0.016, n = 10; Figure 3A on page 16). This association remained significant when creating a sample-specific distribution of the correlation through permutation analysis (rho cut-off = 0.636, Figure 3B left). We then performed leave-one-out analyses, where we iteratively excluded a single patient from the correlation analysis to see whether this result was driven by individual data point. This yielded significant results in 7 of 10 analyses (Figure 3B middle). Finally, we explored whether micro-scale TDL of the resected region also correlated with macro-scale centrality of other cortical regions. This analysis revealed a significant negative correlation between micro-scale TDL of the resected region and macro-scale fMRI centrality of the ipsilateral paracentral lobule (rho = -0.721, p = 0.024, Figure 3B right), although this result did not survive correction for the 36 correlations tested.

Furthermore, faster micro-scale AP rise speed (1st) was significantly related to greater macro-scale fMRI centrality (rho = 0.539, CI [0.042 0.868], p = 0.041, n = 15; Figure 3C/D). This finding remained in permutation testing (rho cut-off = 0.525), but was mostly non-significant in leave-one-out analyses. Spatially, micro-scale AP rise speed (1st) of the resected area was also significantly associated with macro-scale fMRI centrality of the precentral region (rho = 0.582, p = 0.025). Moreover, there were significant negative relationships with fMRI centrality of the superior parietal region (rho = -0.657, p = 0.010) and temporal pole (rho = -0.611, p = 0.018), although these correlations did not survive correction for multiple comparisons.

The only pairwise correlation that survived correction for the nine pairwise tests performed between micro-scale and macro-scale characteristics of the resected region was the significant positive association between micro-scale AP rise speed (1st) and macro-scale multilayer centrality (rho = 0.964, CI [0.698 1.000], p < 0.001, n = 7; Figure 4A on page 18). Moreover, this

association was robust in permutation and leave-one-out analyses, and was spatially specific, also when not correcting for multiple comparisons.

Since different numbers of patients were available for each pairwise correlation depending on modalities available, we also investigated whether the small subset of patients with complete data showed the same patterns of correlation as the larger but heterogeneous samples. Indeed, Figure 2 also shows the associations of both unilayer and multilayer network indices with micro-scale measures in the same samples of 5 patients (for TDL) and 7 patients (for AP rise speeds). As was the case for the mixed samples, only multilayer centrality and AP rise speed (1st) were significantly correlated in this complete dataset, indicating that patient selection differences between the above described subsamples did not confound the reported pairwise correlations.

From network centrality to memory functioning

Finally, we investigated the links between macro-scale network topology of the resected region and memory functioning. A significant canonical correlation was found between centralities of the resected region and memory functioning (canonical correlation coefficient = 0.234, $F(12,670) = 2.14$, $p = 0.013$), such that greater centrality related to better memory functioning.

Unfortunately, very few patients had complete data across these scales, rendering the pairwise testing of associations greatly underpowered. Only greater fMRI centrality was significantly related to greater RAVLT delayed recall (rho = 0.857, CI [0.373 1.000], $p = 0.024$, $n = 7$; Figure 2).

Figure 2. All pairwise correlations

	fMRI centrality	MEG centrality	multilayer centrality
total dendritic length	**0.758 (0.016) | 10**	0.441 (0.154) | 12	0.300 (0.683) | 5
AP rise speed (1st)	**0.539 (0.041) | 15**	0.025 (0.928) | 17	**0.964 (0.0028) | 7**
AP rise speed (20-40Hz)	0.229 (0.411) | 15	0.407 (0.106) | 17	0.107 (0.840) | 7
complete subgroup total dendritic length	0.700 (0.233) | 5	0.400 (0.517) | 5	0.300 (0.683) | 5
complete subgroup AP rise speed (1st)	0.571 (0.200) | 7	0.357 (0.444) | 7	**0.964 (0.0028) | 7**
complete subgroup AP rise speed (20-40Hz)	0.536 (0.236) | 7	0.464 (0.302) | 7	0.107 (0.840) | 7
WMS verbal	-0.126 (0.697) | 12	0.169 (0.620) | 11	0.500 (0.450) | 5
RAVLT immediate	-0.430 (0.125) | 14	0.473 (0.055) | 17	0.017 (0.982) | 9
RAVLT delayed	**0.857 (0.024) | 7**	0.224 (0.567) | 9	0.500 (1.000) | 3

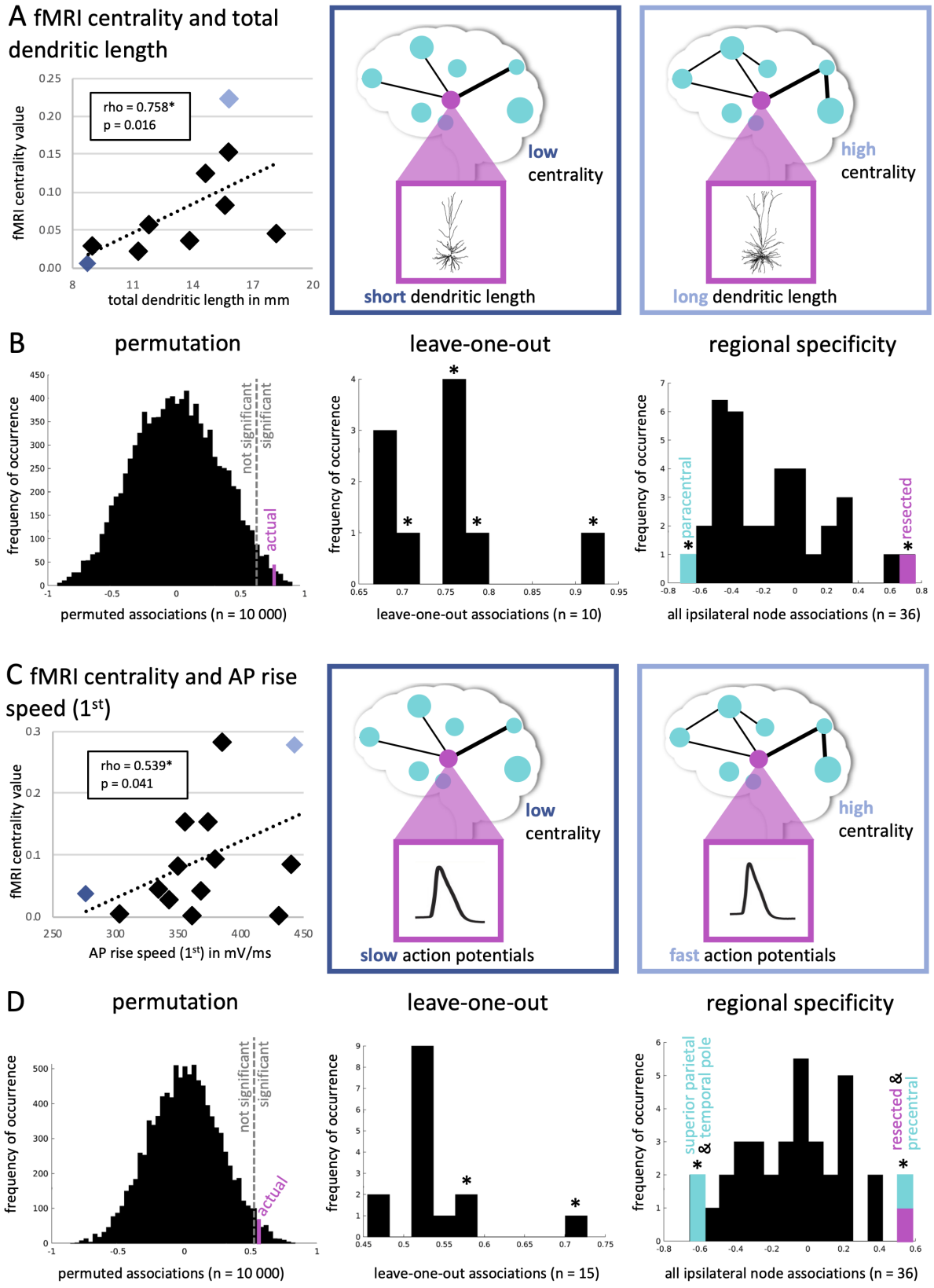
legend

rho (p-value) | n	small negative association
rho (p-value) | n	medium negative association
rho (p-value) | n	small positive association
rho (p-value) | n	medium positive association
rho (p-value) | n	large positive association
rho (p-value) | n	significant positive association
rho (p-value) | n	significant positive association after Bonferroni correction

Legend. This figure shows an overview of all associations between micro-scale properties and macro-scale properties (top three rows), as well as between macro-scale properties and memory functioning (bottom three rows), using the maximum samples of patients with available data. Additionally, the middle three rows (in gray) reflect all pairwise correlations between micro-scale and macro-scale properties when considered in the same subgroup with complete macro-scale data for the morphological (n = 5) and

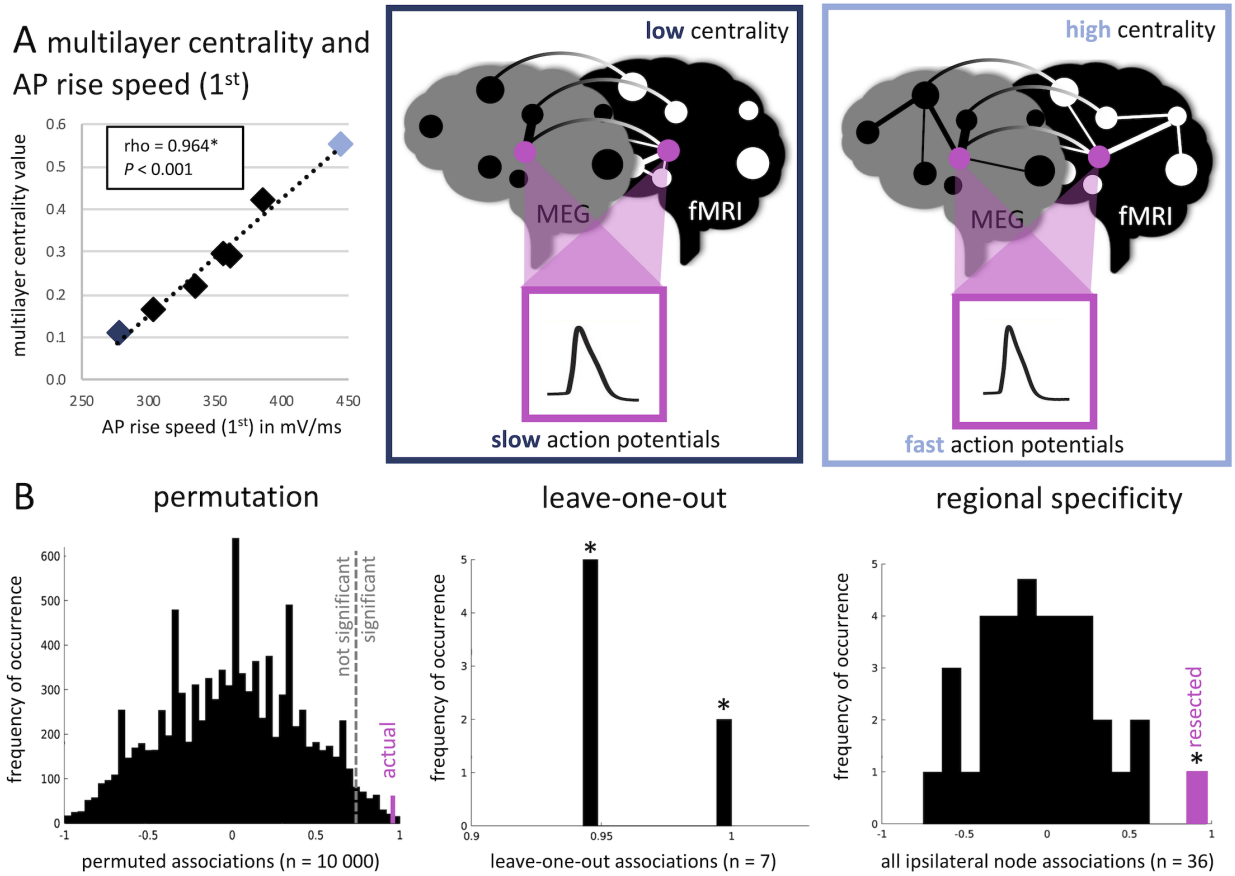
electrophysiological (n = 7) analyses. Green elements reflect positive correlations (with rho, p and n in text), ranging from small correlations (rho < 0.4, light green) to medium correlations (0.4 < rho < 0.6, medium green) to large correlations (rho > 0.6, dark green). White, bold text indicates statistical significance (P < 0.05) and very dark green elements with white, bold text indicates statistical significance after Bonferroni correction for multiple (nine) comparisons. Blue elements reflect negative correlations in the same way. fMRI = functional Magnetic Resonance Imaging, MEG = magnetoencephalography, AP = action potential, WMS = Wechsler Memory Scale, RAVLT immediate = Rey Auditory Verbal Learning Test immediate recall, RAVLT immediate = Rey Auditory Verbal Learning Test delayed recall. In (B), we only select patients with both macro-scale modalities and either of the micro-scale measures available, namely five patients with total dendritic length and seven patients with action potential rise speed. The legend is identical between (A) and (B).

Figure 3. Significant pairwise associations between micro-scale and macro-scale properties



Legend. (A) Displays a scatter plot of the significantly positive correlation between total dendritic length (in mm) and functional Magnetic Resonance Imaging (fMRI) centrality. The dark blue diamond in the plot represents a single patient, who has low centrality and short dendritic length (schematically depicted in the middle panel), while the light blue diamond represents a patient with high centrality and long dendritic length (right panel). In (B), the analyses of the robustness of these results are displayed. The left panel shows the distribution of permuted correlations (10 000 permutations), with the actual (pink) association being smaller than the alpha = 0.05 threshold indicated by the dotted line. The middle panel displays the 10 leave-one-out associations in addition to the real correlation, yielding a significant correlation in 7 of 10 analyses as indicated by the asterisks. The right panel displays all correlations between ipsilateral macro-scale centrality values (n = 36 regions) and total dendritic length of the resected area. The reported positive association (with the resected region) in pink as well as the negative correlation between paracentral centrality and total dendritic length are significant. In (C), a scatter plot of the significantly positive correlation between action potential (AP) rise speed (1st) and fMRI centrality is shown. In (D), the analyses of the robustness of these results are displayed in the same manner as in (B).

Figure 4. Significant pairwise association between micro-scale and macro-scale properties after correction for multiple comparisons



Legend. (A) Displays a scatter plot of the significantly positive correlation between action potential (AP) rise speed (1st) and multilayer centrality, which is the only pairwise association significant after Bonferroni correction for multiple comparisons. The dark blue diamond in the plot represents a single patient, who has low centrality and slow action potentials (schematically depicted in the middle panel), while the light blue diamond represents a patient with high centrality and fast action potentials (right panel). In (B), the analyses of the robustness of these results are displayed. The left panel shows the distribution of permuted correlations (10 000 permutations), with the actual (pink) association being smaller than the alpha = 0.05 threshold indicated by the dotted line. The middle panel displays the 7 leave-one-out associations in addition to the real correlation, yielding a significant correlation in all analyses as indicated by the asterisks.

The right panel displays all correlations between ipsilateral macro-scale centrality values (n = 36 regions) and AP rise speed (1st) of the resected area. The reported positive association (with the resected region) in pink is the only significant correlation.

Discussion

We report on a unique cohort of TLE patients with data spanning multiple scales of investigation. Confirming our hypotheses, individual differences in cellular properties of neurons stemming from the non-pathological lateral temporal lobe correlated with differences in macro-scale functional network centrality of the larger brain region that these neurons were taken from. Patients with longer dendrites and faster action potentials, indication of more integrative potential at the micro-scale, also displayed more integrative profiles of this region in the macro-scale brain network. Finally, variations in network centrality of the resected brain region seemed associated with individual differences in memory functioning.

Previous work has reported that more integratively connected (as measured with diffusion imaging) macro-scale brain regions tend to have bigger neurons with more axons, while locally connected brain regions are made up of smaller neurons that are connected with lower axonal density [30,31]. Interestingly, these studies were performed in postmortem human donors with multiple sclerosis [30] or macaques [31], suggesting that such cross-scale correlations between morphology and network profile may not be specific to TLE, but an evolutionary conserved organizational principle across species. The strength of the current study is that we combined functional and structural micro-scale parameters (action potential kinetics and full morphologies) with functional macro-scale network analysis in the same subjects, an approach not possible in postmortem donors. We found that individuals with more complex neuronal morphology of the lateral temporal lobe (i.e. longer total dendritic length) and faster action potentials also have greater network centrality at that location. Specifically, this association was present between the micro-scale measures and fMRI centrality, while only the relationship between AP rise speed (1st)

and multilayer centrality survived correction for multiple comparisons. This finding suggests that multilayer centrality may capture micro-scale electrophysiological properties of the tissue better than either of the two modalities (fMRI and MEG) alone, which may be in line with other multilayer brain network studies [37,38]. It should, however, be noted that the lack of significance for unilayer network profiles as well as the significance of the multilayer network profile may reflect limited statistical power in these small samples.

We further hypothesized that individual differences in network profiles of the resected DMN region would correlate with memory functioning, which was confirmed by canonical correlation analysis on imputed datasets, corroborating previous work in TLE patients [18–21]. With limited statistical power in pairwise associations, we report only a significant correlation between fMRI centrality and RAVLT delayed recall. Future studies are necessary to assess the specificity of these findings for modalities and memory subtests.

Several limitations of this study should be noted. First and foremost, the uniqueness of this multi-scale dataset meant that only a small sample was available, also precluding subgroup analysis or exploration of confounders that might have affected the current results, e.g. lateralization of the epileptogenic zone, sex, and age. It is also impossible to use a control group for this analysis: healthy individuals can obviously not be subjected to the micro-scale measurements we report on. Another limitation is the spatial resolution of matching between the micro-scale and macro-scale analyses: while certainty about the location of origin of the resected tissue was in the order of millimeters, the atlas region used to reflect the tissue location spans several centimeters. We chose this atlas as it has been used successfully by our group in previous

studies with comparable patient populations and thus has proven cognitive and cellular relevance [35,39–44], but future studies may aim to match tissue locations with increased spatial accuracy.

In conclusion, we show that individual differences in micro-scale cell anatomy and functioning relate to macro-scale network centrality of the temporal lobe in epilepsy patients, the latter of which correlates with memory functioning. These results underline the translational nature of individual differences between epilepsy patients, going from cell via network topology to cognition. Ultimately, a better understanding of these cross-scale individual differences may lead the way to better treatment of neurological disease in general [29], and of memory deficits in TLE patients specifically.

Materials and Methods

Patients

All procedures were performed with the approval of the Medical Ethical Committee of VUmc, and in accordance with Dutch license procedures and the Declaration of Helsinki. Written informed consent was provided by all subjects for data and tissue use for scientific research. Patients did not consent to sharing their raw data. However, the full derived variable set used for statistical analysis in the current work is available from GitHub (https://github.com/multinetlab-amsterdam/projects/tree/master/multiscale_integration), where our code can also found.

Single cell electrophysiology and morphology

Upon surgical resection, the cortical tissue was immediately transferred to ice-cold artificial cerebral spinal fluid, then transported to the electrophysiology lab within 15 mins, where neocortical slices (350µm thickness) were prepared (see [23] for details). Whole-cell patch-clamp recordings were made of layer 2 and layer 3 pyramidal neurons and action potentials (APs) were elicited by incrementing step current injections (step size 30–50 pA). Waveforms were sorted according to their instantaneous firing frequency (1/time to previous AP) and AP rise speed was defined as the peak of AP derivative (dV/dt) for all APs from all neurons from each subject. The rise speed of the 1st AP and APs fired at frequencies between 20-40 Hz were used, as these have previously been associated with intelligence [23].

During electrophysiological recordings, cells were loaded with biocytin through the recording pipette. After recording, slices were fixed in 4% paraformaldehyde and cells were revealed with chromogen 3,3-diaminobenzidine (DAB) tetrahydrochloride using the avidin–

biotin–peroxidase method [45]. Neurons were digitally reconstructed using Neurolucida software (Microbrightfield, Williston, VT, USA). Only neurons with virtually complete dendritic structures were included [34,46]. For each neuron, we extracted total dendritic length (TDL) of all basal and apical dendrites and then averaged these data (1 to 10 neurons per patient).

Macro-scale structural imaging

MRI was performed on a 1.5T magnet (Siemens Sonata) and included an anatomical 3D T1-weighted MPRAGE scan (sequence parameters: TR = 2700ms, TE = 5.2ms, TI = 950ms, 1mm isotropic resolution, 176 slices). Imaging processing was performed using FSL5 (http://www.fmrib.ox.ac.uk/fsl). Standard FSL procedures were used to preprocess structural imaging: non-brain tissue was removed from the 3D T1-weighted images using the Brain Extraction Tool [47], and grey and white matter segmentation was performed using FAST [48]. Non-brain tissue was removed and tissue segmentation was performed. To construct each individual's functional brain network, the Automated Anatomical Labeling atlas was used to define 78 cortical regions [49]. This atlas was warped from standard space to native space, and masked with each subject's native grey matter mask.

Functional magnetic resonance imaging

Patients underwent presurgical fMRI for language localization. Previous studies have shown that the intraindividual effect of any task or state on network topology is small in comparison to interindividual differences in network topology [50], indicating that we may use these pseudo resting-state data to extract individual network signatures that largely generalize outside the

specific task performed in this cohort. Others and we ourselves have previously used resting-state analysis on such task data for network analysis in both healthy controls and patients [35,51,52].

Scanning was performed using a standard echo-planar imaging sequence (TR = 2850ms, TE = 60ms, 144 volumes, 3.3mm isotropic resolution, 7min). During the scan, patients performed a language task in which nine volumes of word generation were alternated with nine volumes of rest (imagery of a landscape).

Preprocessing was performed using standard procedures [53], including discarding the first five volumes, motion correction, spatial smoothing, and high-pass filtering. Six regions with low signal quality (mainly orbitofrontal areas) were excluded, leaving 72 cortical regions for analysis. Additionally, ICA-AROMA was applied to minimize the impact of movement [54]. Functional images were co-registered to the anatomical scans [55]. Time series were extracted from the centroids of all regions, after which a 72x72 connectivity matrix per subject was created using Pearson correlation coefficients. Finally, the absolute values of these correlations were used as weighted connectivity. Further details can be found in the Supplementary Information.

Magnetoencephalography

Patients underwent resting-state MEG as part of their presurgical work-up and/or in the setting of scientific research ([32,33]). Interictal eyes-closed recordings were acquired in supine position using a whole-head system (Elekta Neuromag Oy, Helsinki, Finland) with 306 channels inside a magnetically shielded room (Vacuumschmelze GmbH, Hanau, Germany). Data were recorded with a sampling frequency of 1250Hz, filtered online with a 410Hz anti-aliasing filter and a 0.1Hz high-pass filter. The head position relative to the sensors was recorded continuously with head-

localization coils. A 3D digitizer (Fastrak, Polhemus, Colchester, VT, USA) digitized the head-localization coil positions and scalp outline (roughly 500 points) to allow surface matching of the scalp surface points with anatomical MRI.

Three eyes-closed resting-state recordings of typically 15 min each were recorded for clinical analysis of interictal epileptiform activity. Only one recording was analyzed in this study and chosen according to the following criteria with descending priority: (1) consisting of at least 5 minutes of data, (2) displaying the smallest number of artifacts as per visual inspection, and (3) being the earlier dataset of the three recordings.

Further analysis of these data has been extensively described before [33]. Offline spatial filtering of the raw data removed artifacts using the temporal extension of Signal Space Separation (tSSS [56]) using MaxFilter software (Elekta Neuromag Oy; version 2.1), with details and parameter settings as described in [57]. The reconstruction of neuronal sources was performed with an atlas-based beamforming approach, modified from [58]. The time series (virtual electrodes) for each centroid of each atlas region were reconstructed [59]. These time series were then filtered in the theta band (4-8Hz), because of its proven relevance for cognitive functioning in these patients [60,61] and to limit the number of investigated variables in this limited sample.

As a measure of functional connectivity, the phase lag index (PLI) was used [36]. The PLI assesses the phase-relationship between two regions by quantifying the asymmetry in the distribution of instantaneous phase differences between two time series. It is robust against zero-lag phase synchronization due to volume conduction or field spread [62]. This analysis yielded a 78x78 MEG connectivity matrix per patient.

Network analysis

We then created minimum spanning trees (MSTs) to extract the functional backbone of each fMRI and MEG network and allow for comparison between patients and modalities [32,63–65]. The MST is a binary network that connects all nodes in a network without forming cycles while maximizing connectivity strength. Eigenvector centrality was then calculated per brain region. Of note, there is a plethora of network measures that measure integration and segregation, which are highly intercorrelated (see for instance [66]). Instead of using multiple measures in this small sample with limited statistical power, we chose to focus on eigenvector centrality. Eigenvector centrality is a spectral centrality measure, that not only takes into account the number of connections of a node, but also weighs the number of connections of its neighboring nodes [67].

We then used the fMRI and MEG MSTs to construct a two-layer network consisting of the 72 nodes available in both modalities [77], where each region was connected only to itself across the two layers, forming an interconnected binary multiplex. We then calculated multilayer eigenvector centrality per region [78].

All analyses were performed using in-house scripts in Python, in combination with the Brain Connectivity Toolbox [79] implemented in Matlab R2012a (Mathworks, Natick, MA, USA).

Memory functioning

Patients underwent cognitive assessments during presurgical workup as has been reported before [23]. In order to assess verbal memory functioning, in particular because of its relevance both in terms of tissue location in the DMN and incidence of cognitive deficits, the Wechsler

Memory Scale (WMS [80]) and the Dutch version of Rey's Auditory Verbal Learning Test (RAVLT [81]) were selected for analysis in the current work. From the WMS, the composite Verbal Memory Index was used, which has a mean value of 100 with a standard deviation of 15 in a normative, healthy population. From the RAVLT, immediate recall in terms of total correctly encoded words across five trials (15 words each) was quantified, with possible scores ranging between 0-75 words in total. Additionally, delayed recall of the 15 words was assessed after 15mins, yielding an additional outcome measure of memory retrieval. Higher values indicate better memory performance.

Statistical analysis

Statistical analysis was performed using SPSS Statistics (IBM Corporation, Armonk, NY, USA). Cross-scale pairwise associations in non-imputed data were tested using non-parametric Spearman's correlation coefficients with bootstrapping (1 000 samples, 95% confidence intervals (CI)). When significant, robustness was explored by permuting the micro-macro pairs to create a data-specific correlation distribution (10 000 permutations) and by leave-one-out analysis. Spatial specificity of the associations was explored by correlating the micro-scale measure with macro-scale centralities of all other ipsilateral nodes in the network. Pairwise associations between macro-scale centralities and memory were tested using Spearman's correlation coefficients. The threshold for statistical significance was set at two-tailed alpha < 0.05, but we also report significant results after applying Bonferroni correction for multiple comparisons.

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