Supporting Information

Cashdollar et al. 10.1073/pnas.0904823106

SI Results

Experiment 2: Nonconfigural-Relational (non-CR) Delayed-Match-to-Sample (DMS) Load. *Behavioral performance.* A 2×3 repeated-measures ANOVA (distracter $\times$ load) revealed main effects for DMS load on participants' accuracy [$F_{(2,30)} = 26.5$; $P < 0.001$] and their reaction times (RT)s [$F_{(2,30)} = 15.50$; $P < 0.001$]; however, there was no effect of distracter stimuli presented during the delay for accuracy [$F_{(1,15)} = 1.49$; $P = 0.24$] or for RTs [$F_{(1,15)} = 0.50$; $P = 0.49$]. Pairwise comparisons of accuracy on load (two-tailed, mean, SEM) displayed differences between load 1 vs. load 3 [$96.6 \pm 1.1\%$ and $89.1 \pm 2.4\%$, respectively; $t(15) = 3.332$, $P < 0.01$], between load 3 vs. load 5 [$80.3 \pm 1.9\%$, $t(15) = 3.996$, $P < 0.005$], and between load 1 vs. load 5 items [$t(15) = 9.599$, $P < 0.001$]. Additional pairwise comparisons (two-tailed, mean, SEM) showed increased RTs for item load between load 1 vs. load 3 [924.7 ± 90.1 ms and $1,015.3 \pm 77.4$ ms, respectively; $t(15) = -3.772$, $P < 0.005$] and load 1 vs. load 5 ($1,032.7 \pm 78.5$ ms, $P < 0.005$), whereas no RT differences were found between load 3 vs. load 5 [$t(15) = -1.647$, $P = 0.120$]. Posthoc paired t test comparisons (two-tailed, mean, SEM) were performed to ensure that no differences for RTs between foils ($1,034.2 \pm 13.8$ ms) and targets [$1,019.7 \pm 13.1$ ms, $t(153.4) = 9.39$, $P = 0.35$] or between correct ($1,236.9 \pm 28.5$ ms) and incorrect responses [$1,273.4 \pm 34.2$ ms, $t(35.9) = -0.82$, $P = 0.41$] could inherently bias the effect of DMS load demands on RTs.

Methods

Experiments 1–3: MEG Recording and Wavelet Transformation. MEG data for all experiments were recorded using a 275-channel CTF Omega whole head gradiometer system (VSM MedTech) at a 480-Hz sampling rate and 120-Hz low pass filtering. After participants were comfortably seated in the MEG, head localizer coils were attached to the nasion and preauricularly 1 cm anterior to the left and right tragus to monitor head movement during the recording sessions.

All MEG data were preprocessed with Statistical Parametric Mapping software implemented in Matlab (SPM5; Wellcome Trust Centre for Neuroimaging, University College London Institute of Neurology). Data were band pass filtered to 0.5–100 Hz (Butterworth filtered), and sampling rate was reduced to 140 Hz. Data were epoched and then analyzed using continuous single-trial wavelet transformations within the theta band frequency (15 cycle Morlet wavelet with logarithmic scaling between 3 and 8 Hz) using Matlab based in-house software (1). Single-trial transformations were analyzed separately for amplitude and phase coupling for each subject in the experiment.

Theta-phase coupling. To detect functional coupling between sensor groups, a specific subset of wavelets were selected within the theta band (5, 6, and 7 Hz) for analysis. Then using continuous single-trial wavelet transformations on unaveraged data (band pass filtered between 3 and 9 Hz; sampling of every 4th time point), phase differences were calculated before averaging for each time point of each trial, between all possible sensor combinations of unique sensor pairs and then averaged across trials. Phase alignment for each time point and sensor-pair was measured as the length of the unit phase vector across trials divided by the number of trials. This computation yielded a complex value of phase synchronization ranging from 0 to 1 (2). A value of 1 would correspond to perfect phase alignment across trials and a value of 0 to random phase variation across trials.

Synchrony plots were generated on these transformations

contrasting differences in sensor coupling between testing conditions during the delay after correcting for a 500-ms prestimulus baseline (threshold of $P < 0.05$). Sensor groups displaying significant theta synchrony and clustered with at least three other neighboring significant sensors were chosen for further region of interest analysis using for serial related measures t tests (threshold of $P < 0.05$) to see spatial-temporal changes reveal the magnitude of theta coupling as a function of the experimental manipulation during both sample and delay periods. Phase coupling was not analyzed during the probe phase.

Theta amplitudes. To exclude the possibility that condition differences found in the phase-coupling analysis might be due to parallel differences in theta amplitudes, single-trial wavelet transformations were further analyzed using serial related measures t tests (threshold of $P < 0.05$) on identical sensor groups indicated in the phase-coupling results as having significant differences between conditions. Where as if a specific pattern of wavelet frequencies distinguish between conditions in sensor space for the amplitude analysis in the same direction as the phase-coupling results of the same frequency band then amplitude differences may have biased the phase-coupling signal. Only effects that were significant across at least one entire cycle of the corresponding theta frequency were considered to be reliable (e.g., 200 ms for a 5-Hz theta oscillation) (3).

Experiment 2: Non-CR DMS Load. *Participants.* Eleven right-handed healthy subjects (five male; mean age, 23.2; SD 3.9) participated in the experiment.

Stimuli and task design. Sample, foil, and probe stimuli consisted of 742 black and white photographs of indoor or outdoor scenes. Distracter stimuli consisted of 105 black and white photographs of male and female faces with neutral emotional expression selected from the Karolinska Directed Emotional Faces database (4). Presentation of indoor/outdoor and male/female stimuli were counterbalanced across each block and were kept constant across individual trials. All pictures were gray scaled and normalized to a mean gray value of 127 and a SD of 75, set at 300×300 pixels, and shown on a gray background (127 value).

The experiment was a 2×3 factorial design consisting of seven successive DMS blocks with 30 trials per block, resulting in 35 trials per condition. The manipulations in this experiment were sample stimulus load of one, three, or five items presented serially for 1-s duration each. On half of the trials, a face distracter stimuli (1 s) was presented during the delay period (jittered within a 3-s window during the middle of the delay). For the purposes of this article, only the item load manipulation results will be reported. Subjects were instructed to maintain the sample stimuli (one, three, or five items) over a 5-s delay period while fixating on a cross. At probe subjects were presented with a single picture (1 s) and asked to indicate by button press using the index or middle finger of the right hand if the picture was a "match" or "nonmatch" to one of the aforementioned sample stimuli. Targets and foils were randomized and counterbalanced across testing blocks. After which there was a 3.5-s intertrial-interval where subjects were instructed to blink before fixing on the next cue (0.5 s) (Fig. S2).

Experiment 3: CR DMS in Patients with Bilateral Hippocampal Sclerosis (BHS). *Patient groups.* All patients underwent comprehensive clinical whole brain MRI scans including: T1-weighted, proton density, T2-weighted, and FLAIR acquisition protocols. These images were reviewed by two experienced Consultant Neurora-

diologists who found no structural abnormalities other than BHS in the BHS group (for sample T1-weighted images showing isolated bilateral hippocampal atrophy in BHS and normal appearing hippocampi in the LTN cohort, see Fig. S4).

One-way ANOVAs comparing hippocampal volumes of BHS and LTN patients (hippocampal volumes were according to Woermann et al., ref. 5) confirmed bilateral volume differences [right hippocampus: (F(1,9) = 29.64; $P < 0.001$); and left hippocampus: (F(1, 9) = 31.98; $P < 0.001$]. Independent t tests (two-tailed, mean, SEM) confirmed substantial bilateral hippocampal reductions in the BHS group compared with LTN patients [BHS right hippocampal volume (1.834 ± 0.195 cc) vs. LTN right hippocampal volume (2.933 ± 0.089 cc; $t(9) = -5.444$, $P < 0.001$)] and BHS left hippocampal volume (1.502 ± 0.210 cc) vs. LTN left hippocampal volume [2.925 ± 0.150 cc; $t(9) = -5.655$, $P < 0.001$]. Hippocampal volume data were not available for one patient with BHS. Independent t tests (two-tailed, mean, SEM) confirmed that there were no group differences between BHS and LTN patients in regards to age [BHS, 44.3 ± 3.87 years; LTN, 38.33 ± 4.60 years, $t(10) = 0.998$, $P = 0.342$], age of seizure onset [BHS, 13.3 ± 5.16 years; LTN, 14.08 ± 3.17, $t(10) = -0.125$, $P = 0.903$], performance IQ [BHS, 92.00 ± 9.77; LTN, 102.75 ± 6.34, $t(6) = -0.923$, $P = 0.392$], and working memory digit span [scaled scores derived from the sum of strings recited forward and backward; BHS, 9.50 ± 1.06; LTN, 8.75 ± 2.14, $t(8) = 0.350$, $P = 0.735$] (see Table S2).

Stimuli and task design. A slightly modified version of the DMS paradigm used in experiment 1 was used for this study. A number of trials for the CR condition that proved difficult for healthy subjects in experiment 1 were replaced with a slightly easier version to avoid floor effects in the epilepsy patients. Patients were supported when remembering DMS and control task instructions by presenting instructions for button presses on the lower portion of the display screen (i.e., "which picture did you just see: 1. left, 2. right"). No task instructions were present on the screen during delay periods. This experiment also included 40 additional CR and non-CR DMS trials in which after the delay period only blank boxes were presented during probe. These "no probe" trials comprised half of all of the DMS blocks and were presented randomly within each block. These trials were used to test later recognition memory for the sample stimuli without contamination by repetition effects. ≈30–40 min after completing the DMS tasks, participants took a recognition memory test. For this test, subjects were shown images of which 40 were samples in the CR task (no probe trials) and 40 samples in the non-CR task (no probe trials), and 80 were new scenes (foils), which were not presented previously in the experiment. The scenes were presented one every 3 s with a 1-s intertrial interval where participants classified the scenes as "old" or "new" by making one of two button presses. They were instructed that accuracy and reaction times were equally important. Apart from these aforementioned modifications, all other stimuli and timing parameters were identical to experiment 1.

Experiment 4: Delay Period Interference.
Participants. Seventeen right-handed healthy subjects (11 male, 6 female; mean age, 24.5; SD 4.78) participated in the experiment.

Stimuli and task design. The experiment consisted of two blocked DMS working-memory conditions, the CR condition taken from experiments 1 and the high-load (five samples) condition taken from experiment 3. Participants were required to maintain either five scenes (same as the five-item working-memory load condition of experiment 2) or the configural relationships within a single scene (same as the CR DMS condition in experiments 1 and 3) over a 5-s delay period. Experimental parameters, response requirements, and stimulus material were the same as in the original experiments. There were two blocks of 30 trials each, resulting in 60 trials per DMS condition (ordering of block presentation was randomized).

In the five-item working-memory load condition, subjects were instructed to maintain five sample stimuli (1 s each) over a 5-s delay period while fixating on a cross. At probe, subjects were presented with a single picture (4.5 s) and asked to indicate by button press using the index or middle finger of the right hand if the picture was a match or nonmatch to one of the aforementioned sample stimuli. Targets and foils were randomized and counterbalanced across testing blocks. After which there was a 2-s intertrial-interval where subjects were instructed to blink before fixing on the next cue (0.5 s). The CR DMS condition stimulus timing was exactly matched to the CR condition in experiments 1 and 3.
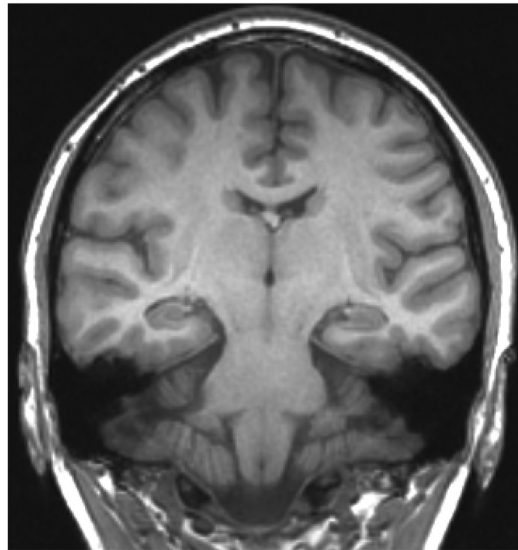
To investigate the effect of interference on the maintenance process for these DMS conditions we presented a difficult visual discrimination task for 3 s during the middle of the 5-s delay period on 50% of the DMS trials. The interference tasks required participants to judge if two scenes presented side-by-side were the same or different (same task as that used in the control condition probes in experiments 1 and 3). This interference task was chosen to disrupt "visual" rehearsal (or replay) by introducing a task that is relevant to the current working-memory maintenance demands (i.e., indoor and outdoor natural scenes).

Behavioral performance. A 2 × 2 within-subjects ANOVA (condition × interference) on the 17 participants tested revealed main effects for condition [F(1,16) = 7.477; $P = 0.015$] and for interference [F(1,16) = 22.535; $P = 0.0001$] with no interaction [F(1,16) = 2.212; $P = 0.156$]. Accuracy was significantly decreased in both DMS conditions when the interference task was presented during delay periods. The five-item load DMS condition performance (83.00 ± 2.45%) was significantly reduced when the interference task was introduced during the delay period [76.24 ± 3.03%, $t(16) = 2.439$, $P = 0.027$]. Similarly, the CR DMS condition performance (78.71 ± 2.47%) significantly decreased with the interference task [67.18 ± 2.38%, $t(16) = 5.229$, $P = 0.0001$]. Importantly, no differences were found in the performance of the interference task between DMS conditions [five-item load DMS interference task (73.00 ± 2.17%) and CR DMS interference task (71.00 ± 2.89%, $t(16) = 0.619$, $P = 0.545$)], suggesting that the delay maintenance process was specifically disrupted and that decreases in performance were not attributed to additional difficulties in encoding sample stimuli between conditions. The impairment of DMS performance with task interference in the delay indicates that the CR DMS task as well as the high load DMS condition both required an active maintenance processes akin to working memory (6).

1. Ryan JD, Althoff RR, Whitlow S, Cohen NJ (2000) Amnesia is a deficit in relational memory. *Psychol Sci* 11:454–461.
2. Lachaux JP, Rodriguez E, Martinerie J, Varela FJ (1999) Measuring phase synchrony in brain signals. *Hum Brain Mapp* 8:194–208.
3. Guderian S, Duzel E (2005). Induced theta oscillations mediate large-scale synchrony with mediotemporal areas during recollection in humans. *Hippocampus* 15: 901–912.
4. Lundqvist D, Flykt A, Ohman A (1998) CD.
5. Woermann FG, Barker GJ, Birnie KD, Meencke HJ, Duncan JS (1998) Regional changes in hippocampal T2 relaxation and volume: A quantitative magnetic resonance imaging study of hippocampal sclerosis. *J Neurol Neursurg Psychiatry* 65:656–664.
6. Shrager Y, Levy DA, Hopkins RO, Squire LR (2008) Working memory and the organization of brain systems. *J Neurosci* 28: 4818–4822.

Amplitude (5.9 Hz wavelet)

Fig. S1. Experiment 1. Serial measures *t* test comparisons plotting the mean theta amplitudes over sensor groups (shown on right-side insets) that were identical to sensor groups that displayed significant theta-phase coupling in Fig. 3 (threshold of $P < 0.05$ per time point if present continuously over three successive theta cycles indicated by markings on *x* axis, error bars indicate SEM). There were either no theta amplitude differences (*A* and *C*), or larger theta amplitudes in the non-CR compared with the CR DMS condition (*B*). These results indicate that theta synchrony cannot be explained by corresponding theta amplitude differences between DMS conditions.

Fig. S2. Experiment 2 MEG paradigm. An example of DMS trials where 1 (one-item load), 3 (three-item load), or 5 (five-item load) samples presented serially must be maintained over a delay period to make a match decision at test.

Fig. S3. Serial measures *t* test comparisons of 6-Hz phase coupling on sensor groups that showed significant coupling (Fig. 2) for one-item load (blue) vs. five-item load (red) DMS conditions in experiment 2 (threshold of $P < 0.05$ per time point if present continuously over three successive theta cycles indicated by markings on *x* axis). The one-item load condition increased theta synchrony of frontal and parietal sensors (*A*), whereas the five-item load condition increased theta synchrony of bilateral frontal sensor groups (*B*).

A Left Temporal Lobe Epilepsy 'MRI-negative' for hippocampal atrophy


Patient (LTN): FT 026

B Bilateral Hippocampal Sclerosis
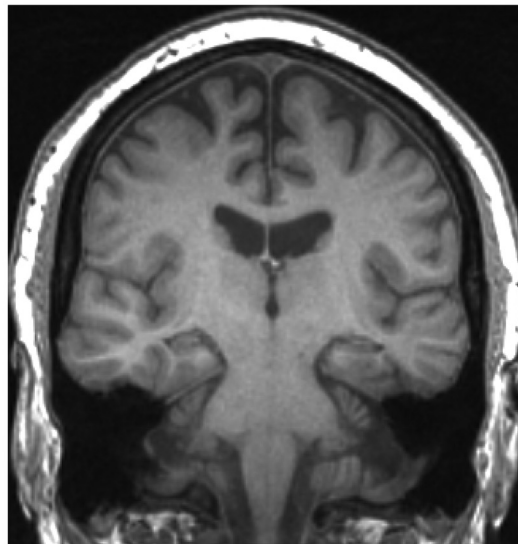
Patient (BHS): TE 015

Fig. S4. Sample T1-weighted images of epilepsy cohorts. (*A*) Patient FT026 with left temporal lobe epilepsy determined to be ''MRI-negative'' for hippocampal volume reductions and signal abnormalities (LTN), and (*B*) epilepsy patient TE015 with isolated BHS and no other apparent structural or signal abnormalities.

A   Non-configural-relational DMS vs Control in BHS
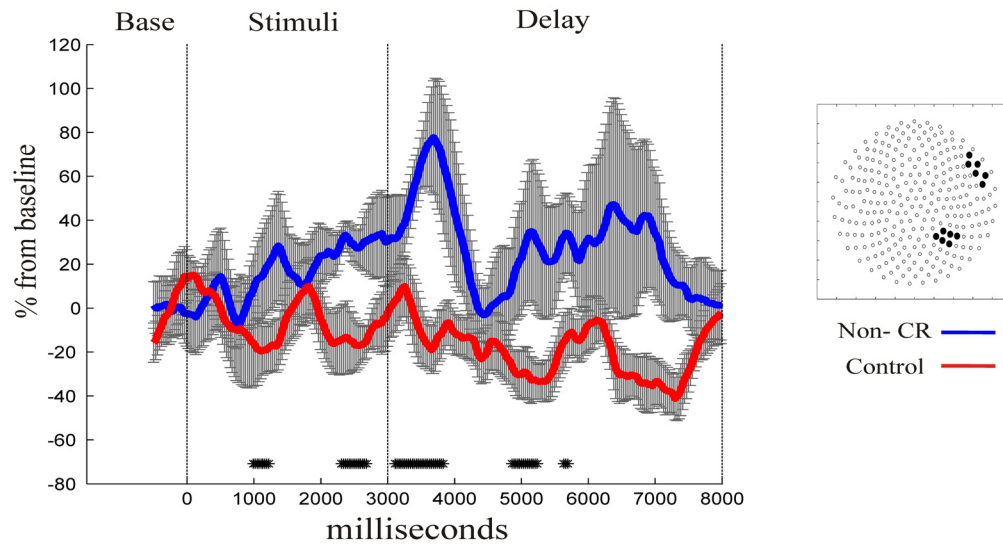
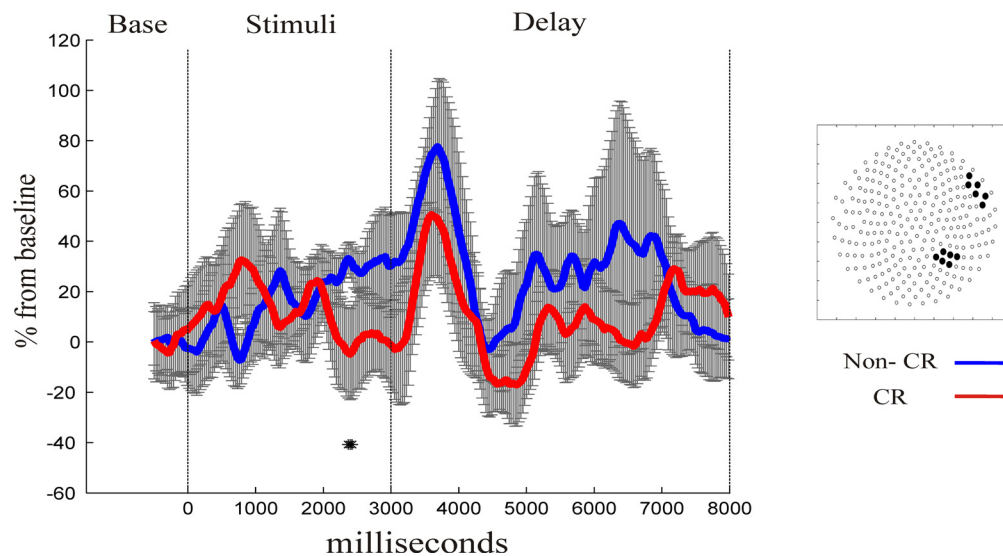## B   Non-configural-relational vs Configural-relational DMS in BHS

Fig. S5. Serial measures *t* test comparisons of 6-Hz phase coupling on analogous sensor groups displayed in Fig. 2 (shown on right-side insets) for non-CR (blue) vs. CR (red) DMS conditions in experiment 3 (threshold of $P < 0.05$ per time point if present continuously over three successive theta cycles indicated by markings on *x* axis). Theta synchrony of frontal and parietal sensor groups is shown to be intact in BHS patients during the non-CR DMS condition (*A*). Comparing non-CR and CR DMS conditions in BHS shows similar spatial-temporal patterns of theta synchrony between frontal and parietal sensor groups (*B*).

Configural-Relational DMS LTN vs BHS
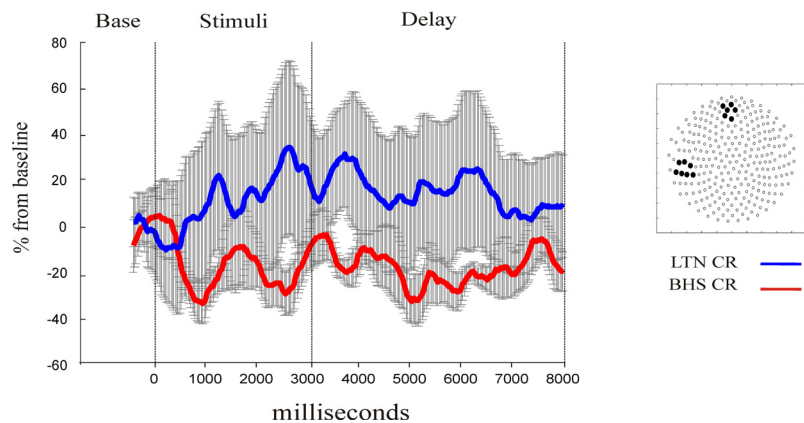

Fig. S6. Serial measures *t* test comparisons of 6-Hz phase coupling on selected sensor groups (shown on right-side insets) during the CR DMS condition in experiment 3. Patients with bilateral hippocampal lesions (BHS shown in red) display decreased fronto-temporal theta synchrony compared with temporal lobe epilepsy patients without hippocampal lesions (LTN shown in blue).

Table S1. Raw accuracy scores for BHS patients, left temporal lobe epilepsy patients determined to be MRI-negative for hippocampal reductions (LTN), and normal controls (NC) on CR and non-CR DMS tasks and corrected hit-rates of delayed recognition test

Subject	Dx	non-CR	CR	Recog_non-CR	Recog_CR
DH006	BHS	0.9750	0.5484	0.2273	0.1717
EM011	BHS	1.0000	0.7500	0.3109	0.4135
GP013	BHS	0.9744	0.4118	0.0731	0.1282
TC016	BHS	1.0000	0.6216	0.1133	0.2448
TE015	BHS	0.8718	0.6750	0.2016	0.0817
m_r	BHS	1.0000	0.4750	0.1123	0.1123
SC005	LTN	1.0000	0.7750	0.4721	0.5971
RG014	LTN	1.0000	0.6500	0.3003	0.2746
SN010	LTN	—	—	0.2026	0.2688
FT026	LTN	1.0000	0.7692	0.4125	0.5494
h_p	LTN	1.0000	0.8500	0.1375	0.2375
NM021	LTN	0.9750	0.7750	0.3320	0.2949
DB	NC	1.0000	0.8500	0.3000	0.6250
GS	NC	0.9750	0.8750	0.3375	0.7875
JL	NC	1.0000	0.9250	0.4125	0.8375
SD	NC	1.0000	0.9000	0.5875	0.7125
YA	NC	1.0000	0.7750	0.3250	0.4750
SR	NC	1.0000	0.7368	0.2539	0.1974
YH	NC	0.9750	0.8250	0.5375	0.5875
VA	NC	0.9750	0.8750	0.2375	0.5125
SA	NC	1.0000	0.7895	0.4375	0.5375
AB	NC	1.0000	0.6750	0.1315	0.3468
DB2	NC	1.0000	0.7692	0.3614	0.3473

Due to a hardware malfunction, no behavioral data was collected for SN010 during MEG recording.

Table S2. Demographic information for BHS patients and left temporal lobe epilepsy patients with normal MRI (LTN)

Subject	Dx	DOB	Volume_R	Volume_L	Seizure onset	Seizure type	Seizure freq	VIQ	PIQ	Digit span, WM	Medications
DH006	BHS	15/02/1962	1.44	0.95	17	cps	3× week	104	87	11	Lev 1,250 mg od; Cbz 200 mg od
EM011	BHS	26/08/1983	2.47	1.8	4	cps	1× month	113	116	12	Pgb 675 mg od; Cbz 1,200 mg od; Ace 500 mg od; Flx 20 mg od
GP013	BHS	31/08/1957	*	*	3	cps	2× month	93	*	11	NaVPA 1,000 mg bd; Phy 450 mg bd; Lev 2,500 mg bd; Clob 10 mg tds
TC016	BHS	21/12/1965	1.83	1.96	10	cps	1× month	84	96	8	Cbz 1,800 mg od; Tpm 200 mg od; Prm 500 mg od
TE015	BHS	08/01/1958	1.42	1.04	9	cps	1–2× month	96	*	10	Lev 1,000 mg bd; Cbz 600 mg bd; Pgb 250 mg bd
M_R	BHS	01/08/1963	2.01	1.76	37	cps & sgs	2–3× week	80	69	5	Cbz 700 mg bd; Pgb 300 mg bd; Lmt 150 mg bd
SC005	LTN	22/04/1980	3.04	3.32	6	cps	1× month	97	106	*	Cbz 1,000 mg bd; Lev 1,000 mg bd; Lmt 150 mg bd
RG014	LTN	24/12/1954	3.02	2.88	21	cps	0–6× month	*	*	*	Cbz 400 mg tds; Prm 250 mg tds; Clon 0.5 mg od
FT026	LTN	15/09/1980	2.68	2.8	5 1/2	cps	2–4× month	102	*	8	Lev 1 g bd; Lmt 300 mg bd
H_P	LTN	06/09/1959	3.27	3.41	11	cps	2–3× day	107	119	12	Lev 1,300 mg bd; Phy 150 mg bd; Prop 40 mg od; Lof 70 mg bd
NM021	LTN	12/02/1974	2.75	2.5	18	cps	1× hour	70	90	3	Lev 1,500 mg bd; Clob 20 mg od
SN010	LTN	20/01/1975	2.84	2.64	23	cps and sgs	1× month	96	96	12	Lmt 200 mg bd; Pgb 50 mg bd

Seizure type: complex partial seizures (cps), generalized seizures (sgs). Digit span (scaled scores derived from the sum of strings recited forward and backward): <5 = impaired; 9–11, mid average; 12–13, high average. Medication: Acetazolamide (Ace), Carbamazepine (CBZ), Clobazam (CLB), Clonazepam (CLN), Lamotrigine (LTG), Levetiracetam (LVT), Lofepramine (Lof), Phenytoin (PHT), Pregabalin (PGB), Primidone (PMD), Propranolol (Prop), Sodium Valproate (VPA), and Topiramate (TPM).
*Note some data missing.