



Structural and functional reorganization of contralateral hippocampus after temporal lobe epilepsy surgery

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ABSTRACT

Objective: To explore the structural and functional reorganization of contralateral hippocampus in patients with unilateral mesial temporal lobe epilepsy (mTLE) who achieved seizure-freedom after anterior temporal lobectomy (ATL).

Methods: We obtained high-resolution structural MRI and resting-state functional MRI data in 28 unilateral mTLE patients and 29 healthy controls. Patients were scanned before and three and 24 months after surgery while controls were scanned only once. Hippocampal gray matter volume (GMV) and functional connectivity (FC) were assessed.

Results: No obvious GMV changes were observed in contralateral hippocampus before and after successful surgery. Before surgery, ipsilateral hippocampus showed increased FC with ipsilateral insula (INS) and temporoparietal junction (TPJ), but decreased FC with widespread bilateral regions, as well as contralateral hippocampus. After successful ATL, contralateral hippocampus showed: (1) decreased FC with ipsilateral INS at three months follow-up, without further changes; (2) decreased FC with ipsilateral TPJ, postcentral gyrus and rolandic operculum at three months, with an obvious increase at 24 months follow-up; (3) increased FC with bilateral medial prefrontal cortex (MPFC) and superior frontal gyrus (SFG) at three months follow-up, without further changes.

Conclusions: Successful ATL may not lead to an obvious structural reorganization in contralateral hippocampus. Surgical manipulation may lead to a transient FC reduction of contralateral hippocampus. Increased FC between contralateral hippocampus and bilateral MPFC and SFG may be related to postoperative functional remodeling.

1. Introduction

Mesial temporal lobe epilepsy (mTLE) is the most common type of medically intractable epilepsy in adults (Kwan et al., 2011). Mesial temporal structures, especially the hippocampus, play a key role in the generation and propagation of epileptic activities. Hippocampal sclerosis (HS) is the most common pathology underlying refractory mTLE (Malmgren and Thom, 2012). As a well-established therapeutic option for refractory mTLE, anterior temporal lobectomy (ATL) contributes to a favorable seizure-freedom rate of approximately 60–70% (Wieser et al.,

2001). However, epilepsy surgery remains underutilized, with an average delay of 20 years from initial diagnosis to surgery (Haneef et al., 2010; Caciagli et al., 2017). Furthermore, about 30–40% patients continue to have seizures after surgery, even with obvious evidence of unilateral HS. Up to now, it remains difficult to predict the seizure and cognitive outcomes following temporal lobe epilepsy surgery.

Many patients with unilateral mTLE also showed significant contralateral interictal and ictal epileptiform activity during scalp and invasive electroencephalogram (EEG) recordings, which was thought to be related to surgical outcome and memory impairment (Passarelli et al.,

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2015; Jehi et al., 2010; Pinto et al., 2017). Does abnormal ipsilateral hippocampus affect the contralateral hippocampus which seemed normal? Do these effects disappear with seizures after successful surgery? What is the role of the contralateral hippocampus in postoperative structural and functional reconstruction? All these questions remain unclear and need to be solved.

Some study found no significant difference between patients and healthy controls in postoperative gray matter volume (GMV) of contralateral hippocampus (Noulhiane et al., 2006), while most studies found a degree of contralateral hippocampal atrophy at some point after TLE surgery when compared with preoperative data (Araujo et al., 2006; Fernandes et al., 2014). Furthermore, the atrophy may be progressive lasting from days to years, which might be related to the deafferentation caused by surgical dissection of bilateral hippocampal connections (Elliott et al., 2016). However, this longitudinal change was observed in only ten TLE patients with different seizure outcomes after different surgical procedures. The dynamic structural changes of contralateral hippocampus after surgery, especially after successful surgery, was still not well known.

Besides, the hippocampus contralateral to the epileptogenic focus in mTLE showed abnormal functional connectivity (FC) with widespread brain regions: significantly reduced connections with ipsilateral temporal lobe and bilateral ventral prefrontal cortex, which may be related to emotional and cognitive changes in mTLE patients (Trotta et al., 2013); increased connection with posterior cingulate cortex (PCC), which was thought to be associated with the memory function before and after surgery (McCormick et al., 2013). Furthermore, it was reported that network integration in contralateral temporoparietal region was associated with surgical outcome in TLE (DeSalvo et al., 2020). However, dynamic changes of contralateral hippocampus after TLE surgery were not well researched. Limited evidence indicated increased activations of contralateral hippocampus during memory-encoding tests at 3 and 12 months after ATL when compared with preoperative data (Sidhu et al., 2016). In that study, the compensatory hippocampal recombination at 3 months was thought to be short and inefficient, while the recombination at 12 months was thought to be effective and contribute to postoperative memory outcomes. Morgan and his colleagues found decreased contralateral hippocampal FC after surgery, which were thought to be a reflection of ongoing epileptogenic progression altered by the surgery, not the direct result of surgery, and may contribute to long-term seizure outcome (Morgan et al., 2019). Therefore, it is important to reveal the dynamic changes of contralateral hippocampus before and after surgery, which may indicate postoperative seizure and cognitive outcomes, as well as structural and functional reorganizations.

We previously explored the longitudinal diffusion and amplitude of low-frequency fluctuations (ALFF) alterations in mTLE patients and revealed postsurgical structural and functional reorganizations from the whole brain level (Li et al., 2019, 2021). In this study, we focused on the key structure of mTLE and assessed the longitudinal GMV and FC changes of contralateral hippocampus in mTLE patients who achieved seizure-freedom after ATL for at least two years. Since hippocampal sclerosis (HS) is the most common pathology in mTLE, we only included mTLE patients with HS or normal MRI. Preoperative GMV and FC could tell us potential structural and functional abnormalities of contralateral hippocampus which seemed normal. The dynamic GMV and FC changes after successful surgery could answer whether these abnormalities disappear with seizure remission and help us further understand the role of contralateral hippocampus in structural and functional plasticity in mTLE.

2. Methods

2.1. Participants

Patients who underwent ATL in West China Hospital due to intractable mTLE were originally included from April 2014 to October 2018.

All of them underwent comprehensive multidisciplinary preoperative evaluations, combining the ictal semiology, ictal and interictal EEG, MRI and PET/CT if available, to localize the seizure focus. Seizure outcomes were evaluated according to the ILAE classification (Wieser et al., 2001) every three months after surgery. High-resolution 3D T1 and resting-state functional MRI (rs-fMRI) data was obtained before and three and 24 months after surgery.

The inclusion criteria were as follows: (1) patients with intractable mTLE according to the International League Against Epilepsy (ILAE) Classification Schemes of Epileptic Seizures and Epilepsy Syndromes (Engel, 2001); (2) normal MRI or with evidence of hippocampal sclerosis (HS) concordant with EEG findings; (3) no evidence of bilateral hippocampal sclerosis or of a secondary extrahippocampal lesion that may contribute to seizures (Liu et al., 2021); (4) reached favorable outcome (ILAE class 1) for at least two years after ATL.

The exclusion criteria included: (1) patients with any other neurological disorder, psychiatric disorder or serious systemic disease; (2) with alcohol or other substance abuse; (3) with other structural lesions except HS according to ILAE classification (Blümcke et al., 2013) confirmed by postoperative histopathological examination; (4) suffered persistent postoperative seizures; (5) lost to follow-up, clinically or radiologically.

This study was approved by West China Hospital ethics committee and informed consent was obtained from all participants.

2.2. Image acquisition

MRI imaging data was acquired on a 3.0 T MRI system (Tim Trio; Siemens, Erlangen, Germany) with an eight-channel head coil at West China Hospital. Participants were instructed to rest with their eyes closed and keep their heads still. Foam pads were used in the scanning procedures to reduce head motion. High-resolution T1 MRI was acquired using a 3D magnetization prepared rapid acquisition gradient echo (MPRAGE) sequence with the following parameters: repetition time (TR): 2300 ms; echo time (TE): 4.18 ms; flip angle: 9°; field of view (FOV): 256 × 256 mm²; voxel size: 1.0 × 1.0 × 1.0 mm³. An echo-planar imaging (EPI) sequence was used to obtain the rs-fMRI data as follows: repetition time/echo time (TR/TE): 2000/30 ms; flip angle: 90°; slice thickness: 3 mm (no slice gap); matrix: 64 × 64; FOV: 240 × 240 mm²; voxel size: 3.75 × 3.75 × 5 mm³; 30 axial slices; and 200 image volumes.

2.3. Hippocampal volumetry estimation

To characterize the regional volume of the hippocampus, structural images were processed with the Computational Anatomy Toolbox (CAT) within SPM12 (<http://www.neuro.uni-jena.de/cat/>). A fully automated processing known as region-based morphometry (RBM) was performed. CAT12 allows estimation of regional tissue volumes for different volume atlas maps. The idea of this approach is that regions of interest (ROIs) can be defined once in an atlas brain and can be then mapped to the individual brain by using a high-dimensional spatial registration. The bilateral hippocampal ROIs were defined using an identified atlas (Hammers et al., 2003). Using the RBM, the hippocampal gray matter volumetry was estimated in native space.

2.4. Surgical resection

SPM12 (www.fil.ion.ucl.ac.uk/spm) was applied for imaging processing. First, T1 images of right-sided TLE (nine patients) were flipped from left hemisphere to right hemisphere to obtain contralateral and ipsilateral datasets. The preoperative T1 images (28 patients) were registered to a symmetric template in MNI space by a non-linear transformation. According to previous studies (Zhang et al., 2017), the symmetric template was obtained by averaging the MNI template and its left-right flipped counterpart. The normalization generated a non-linear transformation function from native space to MNI space. Second, in

subject's native space, the surgical resection of each TLE was manually delineated for mask by overlaying the postoperative T1 images on the preoperative T1 images. The final decision was based on the agreement of two independent investigators. Third, the surgical resection masks and postoperative T1 images were first registered to the corresponding preoperative T1 images using affine registration, and then normalized to the symmetrical template using the nonlinear transformation function. This generated surgical resection masks of each patient in MNI space. These masks were averaged to create a group-level resection mask to remove voxels within resection.

2.5. fMRI data processing

The resting-state fMRI preprocessing was conducted using SPM12 and DPABI (<http://rfmri.org/dpabi>). First, fMRI data of right-sided TLE (nine patients) were left-to-right flipped. In addition, to avoid potential bias in comparisons between TLE and HC, a sub-sample of HC (nine controls), which age- and gender- matched with right-sided TLE patients, was selected to also perform a hemisphere flipping according to an in-house group matching algorithm (Supekar et al., 2019). The preprocessing steps included: (1) discard first five time points. (2) Slice-time correlation. (3) Spatial realignment correlation. (4) normalized to a symmetric MNI template and re-sample to $3 \times 3 \times 3 \text{ mm}^3$. (5) regressing out nuisance covariates including 24 motion parameters and WM and CSF signals. (6) Smoothing only in a mask without the resection areas with a Gaussian kernel (full-width at half-maximum = 6 mm). Participants with head motion exceeding 1 mm or 1° were excluded. During the normalization, preoperative functional images from individual native space were nonlinearly registered to a symmetrical MNI template, yielding transformation functions $f(x)$. Postoperative images were registered to the corresponding preoperative images using affine registration to reduce possible stretching effect and then nonlinearly normalized to the symmetrical template using the same transformation functions $f(x)$ with the preoperative images. This normalization method is similar with our previous DTI study exploring longitudinal diffusion alterations after TLE surgery (Li et al., 2019).

A seed-based FC analysis was used to estimate the hippocampal FC. Based on a verified atlas (Fan et al., 2016), four regions of bilateral rostral and caudal hippocampus were extracted as the seeds. Pearson's correlation coefficients were calculated between time series of each seed and other voxels in the whole brain, and then transformed into Fisher's z-scores. For preoperative TLE and HC, four FC maps were obtained. However, for these postoperative TLE, only the two FC maps with contralateral hippocampus as seeds were obtained. In addition, a resection mask was applied in calculation of the postoperative FC maps to remove the surgical areas.

2.6. Statistical analysis

Independent two-sample *t*-test was used to compare the differences of GMV and FC maps between preoperative TLE and HC with age, gender and intracranial volume as covariates. Paired sample *t*-tests were used to compare the longitudinal GMV and FC changes of contralateral hippocampus between pre- and postoperative data, with age, gender, intracranial volume and resection volume as covariates.

As the ipsilateral hippocampal seeds have been resected, only the FC between contralateral seeds and whole brain regions outside the resection mask were extracted. Paired sample *t*-tests were used to compare the longitudinal FC changes between baseline (before surgery) and 3-month follow-up scan. For those FC exhibiting changes at 3-month follow-up scan were further analyzed to characterize the dynamic changes by comparing the differences between three months and 24 months after surgery using paired sample *t*-tests. As for the voxel-wise FC comparisons, multiple comparisons correction was performed using a voxel threshold ($P < 0.001$) and a cluster threshold estimated by Gaussian Random Field theory (FWE corrected $P < 0.05$).

Furthermore, we adopted mixed linear models (pre vs, 3 m post vs. 24 m post) for GMV and FC multiple comparisons within patients. We also adopted a secondary analysis of GMV and FC comparison between left and right TLE patients, as well as between HS and none-HS patients.

3. Results

3.1. Participants

Finally, 28 patients (12 males) were enrolled. The mean age at surgery was 25.9 years old (range: 15–44 years old). The mean duration of epilepsy was 13 years (range: 2–31 years). All patients had at least one seizure per month before surgery, with or without evolution to bilateral tonic-clonic seizures. Most patients (25/28, 89.3%) only demonstrated interictal epileptic discharges ipsilateral to the side of surgery except three had independent bilateral EEG abnormalities before surgery. Seven patients had normal MRI while the other 21 had evidences of hippocampal sclerosis concordant with EEG findings. All patients underwent unilateral ATL (19/9, L/R) and achieved ILAE class 1 after surgery for at least two years, with a median follow-up time of 35 months (range: 27–52 months). Antiseizure drugs in the first two years after surgery remained the same as those prior to surgery for all patients. Hippocampal sclerosis (HS) was confirmed by postoperative histopathological test in 17 patients and gliosis only was found in the other 11 patients. All patients were scanned before surgery. Thereinto, 19 patients completed the 3-month follow-up scan and 24 patients completed the 24-month follow-up scan. Detailed demographic and clinical information of patients were summarized in Table 1.

Twenty-nine healthy controls (13 males), with a mean age of 27.5 years old (range: 19–42 years old) were enrolled. They were only scanned once with the same protocol as the patients. There was no statistical difference in age and gender between the patients and control group. All participants were native Chinese speakers and right-handed assessed by the Edinburgh Inventory handedness test.

3.2. GMV changes of bilateral hippocampi before and after surgery

Before surgery, GMV of ipsilateral hippocampus in mTLE patients was significantly smaller than that in healthy controls ($P < 0.005$), while there was no statistical difference in contralateral hippocampus between patients and controls. After successful ATL, no obvious GMV changes were observed in contralateral hippocampus between pre- and 3 m- and 24 m-postoperative scans (Fig. 1). Detailed GMV information of all patients at all three timepoints was showed in the supplementary material (Fig. S1).

Furthermore, no GMV differences were found in contralateral hippocampus between left and right TLE patients, as well as between HS and none-HS patients at any timepoint. Details of GMV changes in each group were showed in the supplementary material (Fig. S2).

3.3. FC changes of bilateral hippocampi before surgery

Regions showing altered FC with bilateral hippocampi before surgery were listed in Table 2.

As showed in Table 2 and Fig. 2, before surgery, ipsilateral rostral hippocampus (rosHIP) showed increased FC with ipsilateral insula (INS) and decreased FC with ipsilateral posterior cingulate cortex (PCC), contralateral angular gyrus (AG) and bilateral cerebellum posterior lobe (CPL). Ipsilateral caudal hippocampus (cauHIP) showed increased FC with ipsilateral INS and temporoparietal junction (TPJ) and decreased FC with ipsilateral thalamus, PCC; contralateral hippocampus, AG; bilateral CPL, superior frontal gyrus (SFG), caudate and medial prefrontal cortex (MPFC). Contralateral rosHIP showed decreased FC with ipsilateral hippocampus and contralateral AG. Contralateral cauHIP showed decreased FC with ipsilateral hippocampus.

Table 1

Demographic and clinical characteristics of mTLE patients.

Subject	Sex	Age at surgery (year)	Disease duration (years)	Seizure type	Seizure frequency	Distribution of IEDs	Seizure focus	Preoperative MRI finding	MRI follow-up
TLE_01	M	22	14	FS	Monthly	L	L	normal	pre/24m
TLE_02	F	22	10	FS, sGTCS	Weekly	L, R	L	HS	pre/24m
TLE_03	F	26	17	FS	Weekly	L	L	HS	pre/24m
TLE_04	F	35	31	FS, sGTCS	Monthly	L	L	normal	pre/24m
TLE_05	M	20	4	FS, sGTCS	Weekly	L	L	HS	pre/3m/24m
TLE_06	F	25	15	FS, sGTCS	Monthly	L, R	L	HS	pre/3m/24m
TLE_07	F	26	6	FS, sGTCS	Daily	L	L	HS	pre/3m/24m
TLE_08	M	22	10	FS	Daily	R, L	R	HS	pre/3m
TLE_09	F	41	10	FS	Weekly	R	R	HS	pre/3m/24m
TLE_10	M	17	8	FS, sGTCS	Monthly	L	L	HS	pre/24m
TLE_11	M	20	14	FS, sGTCS	Monthly	R	R	HS	pre/3m/24m
TLE_12	F	38	27	FS	Monthly	R	R	normal	pre/3m/24m
TLE_13	F	19	12	FS	Weekly	R	R	HS	pre/3m/24m
TLE_14	M	28	21	FS, sGTCS	Monthly	L	L	normal	pre/3m/24m
TLE_15	M	18	4	FS	Monthly	L	L	HS	pre/3m/24m
TLE_16	M	20	19	FS, sGTCS	Monthly	L	L	HS	pre/3m/24m
TLE_17	M	22	22	FS, sGTCS	Daily	R	R	HS	pre/3m/24m
TLE_18	M	17	7	FS	Monthly	L	L	HS	pre/24m
TLE_19	M	41	13	FS	Monthly	L	L	normal	pre/24m
TLE_20	F	23	2	FS, sGTCS	Monthly	R	R	HS	pre/3m/24m
TLE_21	F	25	14	FS, sGTCS	Weekly	L	L	HS	pre/3m
TLE_22	F	26	9	FS, sGTCS	Daily	L	L	normal	pre/3m/24m
TLE_23	F	39	21	FS, sGTCS	Weekly	R	R	HS	pre/24m
TLE_24	F	33	18	FS	Monthly	R	R	HS	pre/3m/24m
TLE_25	F	15	3	FS, sGTCS	Weekly	L	L	HS	pre/3m/24m
TLE_26	F	19	18	FS, sGTCS	Monthly	L	L	normal	pre/24m
TLE_27	F	22	14	FS, sGTCS	Monthly	L	L	HS	pre/3m
TLE_28	M	44	15	FS, sGTCS	Monthly	L	L	HS	pre/3m

TLE: temporal lobe epilepsy; IEDs: interictal epileptic discharges; ILAE: International League Against Epilepsy; M: male; F: female; FS: focal seizure; sGTCS: secondary generalized tonic-clonic seizure; L: left; R: right; HS: hippocampal sclerosis; pre: preoperative; 3 m: 3 months after surgery; 24 m: 24 months after surgery.

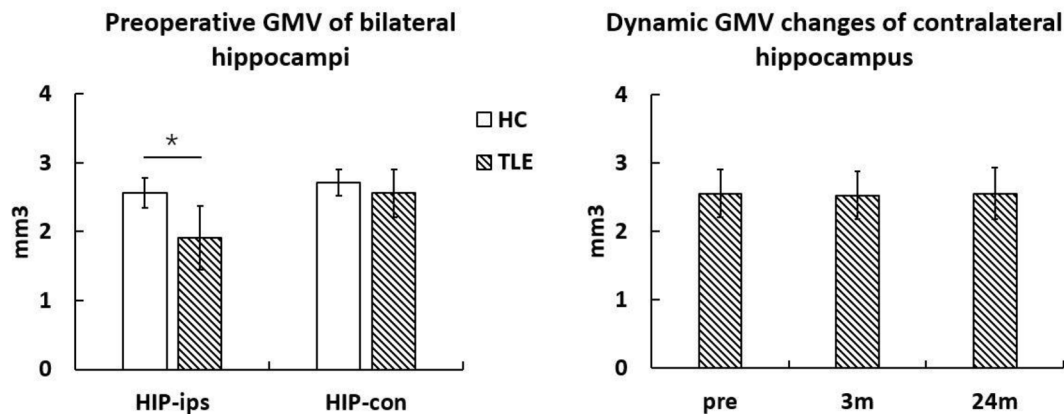


Fig. 1. GMV Changes of bilateral hippocampi before and after surgery. HIP-ips: ipsilateral hippocampus; HIP-con: contralateral hippocampus; HC: healthy controls; TLE: temporal lobe epilepsy; pre: preoperative; 3 m: 3 months after surgery; 24 m: 24 months after surgery. * $P < 0.005$.

3.4. FC changes of contralateral hippocampus after surgery

Dynamic FC changes of contralateral hippocampus after surgery

were showed in Fig. 3.

After successful ATL, contralateral rosHIP showed: (1) FC decrease with ipsilateral INS and contralateral superior temporal gyrus (STG) at 3

Table 2
Regions showing altered FC with bilateral hippocampi before surgery.

Seed and Brain Regions	Peak MNI Coordinates			T Value	Cluster Size (Voxels)
	X	Y	Z		
<i>rosHIP-ips</i>					
Cerebellum Posterior Lobe-ips	-24	-72	-33	-5.5	299
Cerebellum Posterior Lobe-con	12	-84	-39	-4.5	184
Insula-ips	-39	12	-9	4.35	107
Posterior cingulate cortex-ips	-3	-42	30	-4	114
Angular Gyrus-con	-51	-63	39	-4.3	139
<i>cauHIP-ips</i>					
Cerebellum Posterior Lobe-ips	-21	-84	-30	-6.7	728
Cerebellum Posterior Lobe-con	42	-72	-36	-5.5	507
Hippocampus-con	24	-36	-3	-5.9	331
Thalamus-ips	-3	-12	0	-4.5	331
Insula-ips	-39	3	-9	5.48	199
Medial prefrontal cortex	0	57	5	-7.3	1790
Superior frontal gyrus-con	23	33	48	-4.5	1790
Superior frontal gyrus-ips	-21	37	47	-5.5	1790
Caudate-con	9	12	3	-5.1	254
Caudate-ips	-12	15	3	-6.5	210
Posterior cingulate cortex-ips	0	-42	24	-6.9	1211
Angular Gyrus-con	54	-60	33	-5.2	227
Temporoparietal junction-ips	-57	-33	33	4.48	123
<i>rosHIP-con</i>					
Hippocampus-ips	-27	-12	-21	-4.8	182
Angular Gyrus-con	48	-54	48	-4.9	131
cauHIP-con Hippocampus-ips	-30	-27	-15	-5.7	181

MNI: Montreal Neurological Institute; rosHIP: rostral hippocampus; cauHIP: caudal hippocampus; ips: ipsilateral; con: contralateral.

months follow-up when compared with preoperative scan, without further changes when compared with 24 months follow-up scan; (2) FC decrease with ipsilateral TPJ and postcentral gyrus (PCG) at 3 months follow-up, with FC increase at 24 months follow-up.

Contralateral cauHIP showed: (1) decreased FC with ipsilateral INS at 3 months follow-up, without further changes at 24 months follow-up; (2) decreased FC with ipsilateral TPJ and rolandic operculum (RO) at 3 months follow-up, with increased FC at 24 months follow-up; (3) increased FC with bilateral MPFC and SFG at 3 months follow-up, without further changes at 24 months follow-up.

In addition, ipsilateral inferior temporal gyrus (ITG), caudate and RO showed significant FC decrease with contralateral hippocampus after successful ATL through mixed linear models (pre vs. 3 m post vs. 24 m post). Details of these regions were showed in the supplementary material (Table S1). No FC increase was found between pre- and post-operative data.

3.5. Secondary analysis between different subgroups

We didn't find any FC differences of contralateral hippocampus between left and right TLE patients, as well as between HS and none-HS patients. Details of FC changes in each group were showed in the supplementary material (Figs. S3 and S4).

4. Discussion

In the current study, we explored the structural and functional reorganization of the contralateral hippocampus before and after ATL in patients with unilateral mTLE who achieved seizure-freedom for at least two years. Before surgery, ipsilateral hippocampus showed significant atrophy, increased FC with ipsilateral INS and TPJ and decreased FC with widespread bilateral regions, as well as contralateral hippocampus. Contralateral hippocampus was structurally normal before surgery, without obvious GMV changes at three and 24 months after surgery. After successful surgery, contralateral hippocampus demonstrated: (1) decreased FC with ipsilateral INS at three months follow-up, without further changes; (2) decreased FC with ipsilateral TPJ, PCG and RO at

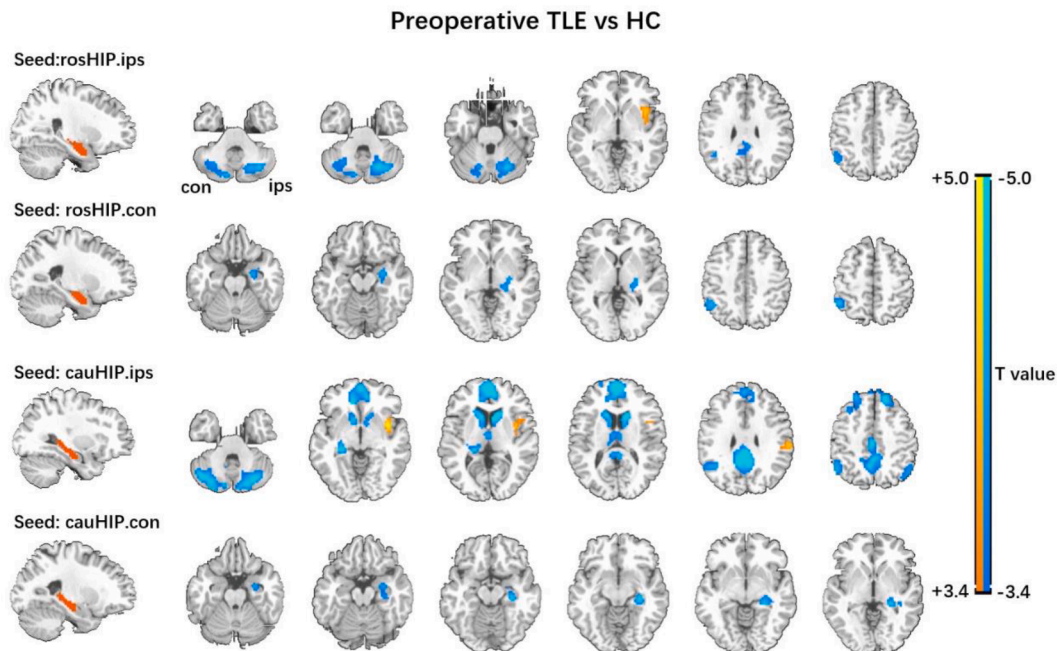


Fig. 2. FC changes of bilateral hippocampi before surgery. Regions with warm colors (positive T values) mean stronger FC in TLE patients compared with controls and regions with cold colors (negative T values) mean the opposite. TLE: temporal lobe epilepsy; HC: healthy controls; rosHIP: rostral hippocampus; cauHIP: caudal hippocampus; ips: ipsilateral; con: contralateral.

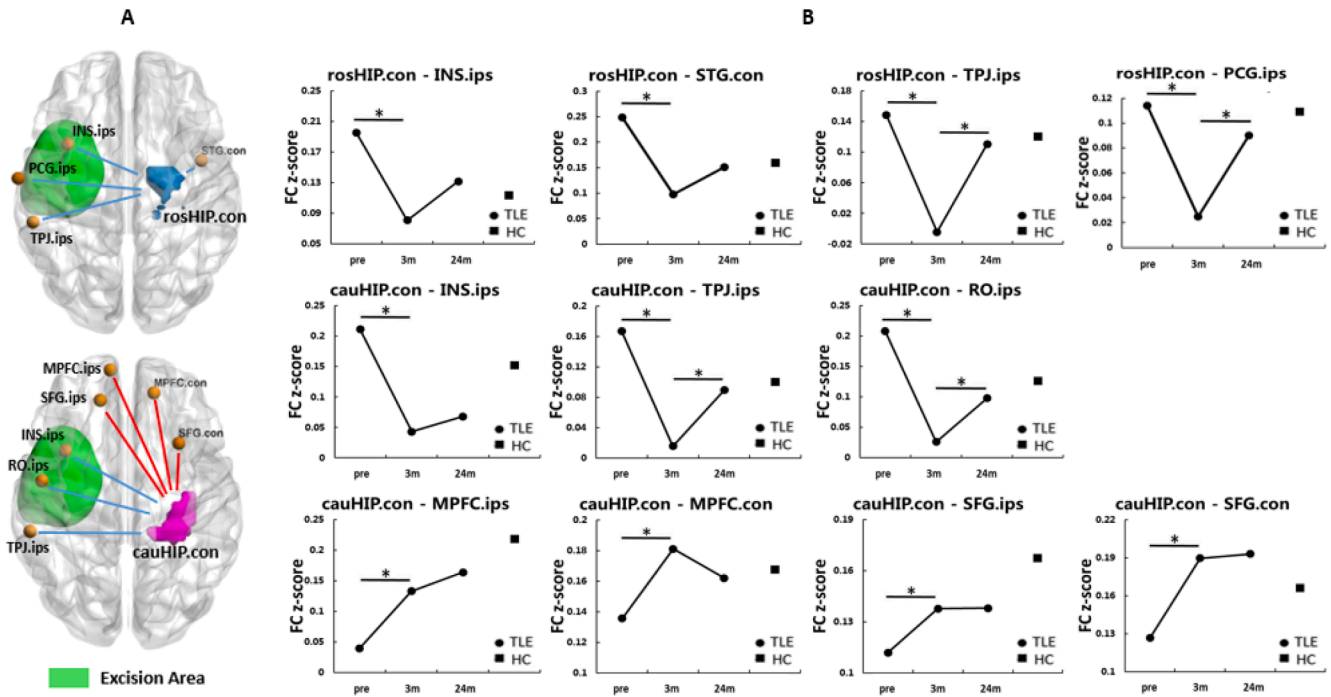


Fig. 3. FC changes of contralateral hippocampus after surgery. **Fig. 3A** represented hippocampal FC changes at 3 months follow-up. Red lines mean increased FC and blue lines mean decreased FC. **Fig. 3B** represented dynamic FC changes between preoperative and 3- and 24-months follow-up. X-axis: different time point, Y-axis: FC z-score. roHIP: rostral hippocampus; cauHIP: caudal hippocampus; ips: ipsilateral; con: contralateral; INS: insula; STG: superior temporal gyrus; TPJ: temporo-parietal junction; PCG: postcentral gyrus; RO: rolandic operculum; MPFC: medial prefrontal cortex; SFG: superior frontal gyrus; TLE: temporal lobe epilepsy; HC: healthy controls; pre: preoperative; 3 m: 3 months after surgery; 24 m: 24 months after surgery. * $P < 0.05$.

three months follow-up, with an obvious FC increase at 24 months follow-up; (3) increased FC with bilateral MPFC and SFG at 3-mon follow-up, without further changes.

4.1. GMV changes of bilateral hippocampi

It was not surprising that ipsilateral hippocampus showed significantly atrophy pre-surgically since only unilateral mTLE patients were enrolled and HS was histopathologically confirmed in 17/28 patients. Contralateral hippocampus did not differ from the healthy controls pre-surgically, and did not differ from preoperative status at 3 months and 24 months after successful surgery either. Postsurgical atrophy of contralateral hippocampus has been confirmed by several studies at different timepoints (Araujo et al., 2006; Fernandes et al., 2014). Atrophy of contralateral hippocampus had also been demonstrated to be progressive, lasting from days to years, and associated with postsurgical outcome, the more atrophy the poorer outcome (Elliott et al., 2016). We only recruited seizure-free patients, which might lead to failure in finding similar atrophy process.

4.2. FC changes of ipsilateral hippocampus

In this study, hippocampus was divided into rostral and caudal sections according to a verified atlas to better disclosure the FC patterns of different subregions of hippocampus (Fan et al., 2016). Furthermore, it was reported that different subregions of hippocampus had different functional connectivity with medial temporal structures and were involved in different memory and cognitive functions (Collin et al., 2015; Vos de Wael et al., 2018).

Before surgery, ipsilateral roHIP and cauHIP consistently showed increased FC with ipsilateral insula. The insula is a multimodal functional network hub, involved in many complex functions, such as language, sensation, auditory, visual, limbic and vestibular functions (Dionisio et al., 2019). The strong connectivity between mesial temporal

structures and insula may be implicated as an extratemporal cause for failure of TLE surgery (Dionisio et al., 2019; Barba et al., 2017). Although all our patients became seizure-free for at least two years after ATL surgery, the increased FC between ipsilateral hippocampus and insula did reflect the influence of ongoing seizures or intrinsic etiology of mTLE on insula. Furthermore, stereo-electroencephalography (SEEG) study confirmed that most TLE seizures spread to the insula, rather than arise from it (Isnard et al., 2004). Thus, the involvement of insula in interictal and ictal discharges may be another reason for the increased FC. Besides, ipsilateral cauHIP showed increased FC with ipsilateral TPJ before surgery, which was also observed before (Trotta et al., 2013). As the neighboring region of mesial temporal structures, ipsilateral TPJ was easily irritated by epileptiform discharges arose from mesial temporal structures, which might contribute to the increased FC between ipsilateral cauHIP and TPJ. Furthermore, before surgery, ipsilateral hippocampus showed obvious FC decrease with widespread regions of DMN, such as ipsilateral PCC, contralateral AG, and MPFC, which is in line with previous studies (McCormick et al., 2013; Pittau et al., 2012; Liao et al., 2011). It is well-known that DMN usually get deactivated during cognitive task or interictal epileptic discharges (Liao et al., 2011; Laufs et al., 2007; Kobayashi et al., 2009; Burianová et al., 2017; Tong et al., 2019; Oyegebile et al., 2019), since DMN participates in maintaining baseline brain activities, relative to spontaneous cognition and environmental monitoring (Buckner et al., 2008). Resting state fMRI studies indicated the damaged FC between ipsilateral hippocampus and DMN may in part lead to the cognitive impairments in mTLE patients (Pittau et al., 2012; Zhang et al., 2010). In addition, ipsilateral cauHIP showed FC decreases with bilateral SFG and caudate. Although these direct FC decreases were not found before, previous studies did reveal less activation of SFG (Klamer et al., 2017) and caudate atrophy (Riley et al., 2011) in TLE patients. Furthermore, as the components of FPN, decreased FC in bilateral SFG and caudate may be related to language and memory impairments in mTLE patients.

4.3. FC changes of contralateral hippocampus

Before surgery, contralateral rosHIP and cauHIP consistently showed decreased FC with ipsilateral hippocampus, which is consistent with previous findings (Liao et al., 2011; Guye et al., 2005). Possible reasons included the metabolic dysfunctions induced by epileptic discharges and underlying structural degeneration. Compared with other studies (Trotta et al., 2013; McCormick et al., 2013; DeSalvo et al., 2020), we didn't find obvious FC changes between contralateral hippocampus and widespread extra-temporal regions except decreased FC with contralateral AG. Since we only enrolled patients with favorable seizure outcomes and very low proportion (3/28) of bilateral EEG abnormalities, it is reasonable to speculate that less involvement of contralateral hippocampus before surgery may be related to better seizure outcome after surgery.

Both contralateral rosHIP and cauHIP showed decreased FC with ipsilateral insula at three months follow-up, without further changes, which indicated a relatively stable functional deterioration after surgery and may be a direct result of surgical dissection of bilateral hippocampal connections. Since ipsilateral insula was in close proximity to the resection, shifts after the resection and contaminations by enlarged sulci or resected voxels may also contribute to the FC changes with ipsilateral insula. We tried to adopt affine registration during the data normalization for postoperative image to reduce these effects. Besides, contralateral hippocampus demonstrated FC decrease with ipsilateral TPJ, PCG and RO at three months follow-up, with an obvious increase at 24 months, suggesting a transient but reversible functional impairment induced by surgical resection, followed by a compensatory functional reorganization in neighboring regions.

In addition, after surgery, contralateral cauHIP showed increased FC with bilateral MPFC and SFG at 3 months follow-up, without further changes. The anatomical and functional connections between MPFC and hippocampus were proved to be crucial in rapid learning and memory consolidation (Euston et al., 2012). Other studies also found the FC between non-pathologic mesial temporal lobe and medial frontal cortex was positively correlated with the delayed recall score in mTLE patients, suggesting potential adaptive changes in contralateral hemisphere to preserve memory function (Doucet et al., 2013). Thus, the reinforced functional connectivity between contralateral hippocampus and MPFC may partly offset the memory impairments induced by chronic epilepsy and the resection of ipsilateral hippocampus, indicating potential memory and cognitive remodeling after successful surgery. Similarly, SFG was also involved in working memory (du Boisgueheneuc et al., 2006). Increased FC between contralateral hippocampus and SFG further confirmed the memory reconstruction after successful ATL. As for the dynamic FC changes of contralateral hippocampus with bilateral MPFC and SFG, we didn't find significant changes between three months and 24 months follow-up, suggesting these functional reorganizations after surgery were persistent and might contribute to postoperative memory outcomes. Unfortunately, cognitive tests were not included in most patients, so we couldn't find out direct correlations between these FC changes and postoperative cognitive outcomes. However, these FC increases did reflect significant FC remodeling following postoperative seizure control, especially with bilateral frontal regions. Similarly, previous structural study only found relative gray matter gain of bilateral frontal cortex after surgery in seizure-free patients (Doucet et al., 2015), suggesting postoperative reconstruction after successful TLE surgery may mostly involve frontal cortex.

Regarding our mixed linear models results, only ipsilateral ITG, caudate and RO showed decreased FC with contralateral hippocampus after successful ATL, without obvious FC increase. Our inter-group imbalance (pre/3m post/24 m post, 28/19/24) and strict multiple comparisons (pre vs, 3 m post vs. 24 m post) may be the reason for not finding as many FC changes as paired sample t-tests results.

Furthermore, we didn't find any GMV and FC differences of contralateral hippocampus between left and right TLE patients, as well

as between HS and none-HS patients through direct comparisons. However, we did observe some differences from the changing curves of different subgroups (Figs. S3 and S4). Our small sample size in right TLE (pre/3m post/24 m post, 9/8/8) and none-HS patients (pre/3m post/24 m post, 7/4/7) may limit the statistical power to find potential differences. Further studies with larger sample size were needed.

4.4. Limitations

We have to address several limitations in our study. Firstly, in view of our relatively small sample size, we right-left flipped images to combine analysis. This may affect the results because of different functions of left and right hemisphere, as well as different manipulations between left-sided and right-sided ATL. Further studies with larger sample size could allow independent analysis in each hemisphere. Secondly, not all patients completed both the three months and 24 months follow-up scan, which may cause inter-group imbalance and limit the statistical power. Thirdly, all the healthy controls were only scanned once. We could not exclude normal variations with time although these variations may be very small. Finally, neuropsychological tests, especially the memory tests, were not included for correlation with our FC remodeling. Further studies enrolling larger group of patients and comprehensive neuropsychological tests were needed.

5. Conclusion

Successful ATL may not lead to an obvious structural reorganization in contralateral hippocampus. Surgical manipulation may lead to a transient but reversible FC reduction of contralateral hippocampus. Increased FC between contralateral cauHIP and bilateral MPFC and SFG may be related to postoperative functional remodeling.

6. Credit author statement

Wei Li, Dongmei An and Dong Zhou formulated the study design. Wei Li and Dongmei An performed the literature search, data analysis, data interpretation and wrote the paper. Yuchao Jiang and Cheng Luo helped data analysis. Yingjie Qin and Baiwan Zhou helped collecting the clinical and neuroimaging data. Du Lei, Dezhong Yao and Qiyong Gong consulted on data analysis and helped data interpretation. Heng Zhang and Ding Lei performed the surgery and helped following up patients.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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