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SHORT COMMUNICATION

Dissociation between diffusion MR tractography density and strength in epilepsy patients with hippocampal sclerosis

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Summary Mesial temporal lobe epilepsy (MTLE) is hypothesized to involve derangement of long-range limbic connectivity, but *in vivo* evidence is lacking. We used diffusion tractography to investigate the relationship between hippocampal atrophy and connectivity in MTLE patients with hippocampal sclerosis (HS). Atrophy was correlated with relatively decreased connectivity density but increased connectivity strength, suggesting that HS is accompanied by relatively sparse but strong connections as measured by diffusion anisotropy.

Introduction

A characteristic radiological finding in many patients with hippocampal sclerosis (HS) is reduced volume of the hippocampus in the diseased compared to the non-diseased hemisphere (Pardoe et al., 2009). These volume changes are related to neuropathological damage including loss of pyramidal neurons, dispersion of granule cells, and axonal sprouting (Magloczky, 2010; Marucci et al., 2010; Bote et al., 2008). Structural differences between hippocampi are widely considered when evaluating treatment options for patients with epilepsy, especially in determining their candidacy for resective surgery. While hippocampal sclerosis is detectable and quantifiable using structural MRI, not much is known about the structural connectivity characteristics of the atrophic hippocampus.

Diffusion-weighted MRI (DW-MRI) can be used to investigate the microstructural characteristics of white matter in the living human brain, including the strength and directionality of water diffusion at each imaging element, which is commonly summarized by the fractional anisotropy (FA) computed from the eigenvalues of the diffusion tensor. Tractography methods can then be applied to reconstruct regional and whole-brain trajectories of fiber pathways. Given the spatial resolution of DW-MRI (~2 mm in most human studies), individual pathways represent tens of thousands of axons, and multiple pathways comprise larger

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fiber bundles or tracts. Tractography algorithms, when applied appropriately and interpreted in the light of known limitations, can yield rich information about white matter organization, including pathway density and pathway strength. Density may be quantified by the number or volume of tractography pathways; strength may quantified by integrating FA in voxels representing the individual pathways. Diffusion anisotropy is influenced both by the degree of myelination and coherence of the axons (Beaulieu, 2002).

In this study, we sought to characterize differences in patterns of white matter connectivity between the atrophic and non-atrophic hippocampi in temporal lobe epilepsy patients with HS. At the outset, we hypothesized that given the sizeable volume differences seen in patients with HS, the density of white matter in the diseased hemisphere would show a corresponding decrease. However, we were also interested in understanding the relationship between pathway density and pathway strength, as assessed by FA integrated along the reconstructed pathways. Both deterministic and probabilistic methods for tractography reconstruction have been developed in the last decade to assess the spatial trajectory of white matter fiber tracts. Deterministic algorithms suffer from a weakness in their ability to model fiber pathways that cross within voxels. Probabilistic methods can overcome this to some extent, but the output is challenging to interpret given that connectivity is not expressed as a binary metric. We took the opportunity to apply both methods of tractography reconstruction to diffusion-weighted MRI data from a group of ten TLE patients with HS and in a group of ten healthy control subjects in order to examine the relationship of connectivity density and strength with hippocampal volume.

Methods

Subjects

Ten patients (mean age 34.6, SD 11.5, 4 females, 2 left handed) with pharmaco-resistant temporal lobe epilepsy, who were candidates for surgical intervention, were enrolled in an IRB-approved imaging study after each provided informed consent. Each had hippocampal sclerosis (2 with left HS; 8 with right HS) determined by neuroradiological interpretation and later by pathological analysis of the surgically resected hippocampus. Ten age-matched subjects with no history of neurological or psychiatric illness (mean age 35.0, SD 14.3, 4 females, 2 left handed) were enrolled as healthy controls.

Image acquisition

All patients and controls underwent a single MR imaging session at 3 Tesla (Philips Intera) prior to any surgical intervention. One high-resolution T1-weighted MRI (TR/TE = 8.4/3.9 ms; FA = 8 degrees; matrix size = 256 × 256; FOV = 240 mm; slice thickness = 1.0 mm thick sagittal slices) and a 32-direction diffusion imaging sequence (high angular resolution, overplus on, TR/TE = 8500/67 ms; FA = 90 degrees; matrix size 128 × 128; FOV = 224 mm; 2 mm thick axial slices, max. *b*-value of 800 s/mm^2) were obtained.

Image processing

Image processing, alignment and visualization were performed with AFNI (Cox, 1996). Each diffusion-weighted image was aligned to the skull-stripped T1 MRI, followed by correction of gradient orientations by the angular motion parameters (Leemans and Jones, 2009) and computation of a diffusion tensor. Automated subcortical segmentation of the T1 MRI with Freesurfer v4.5.0 (Fischl et al., 2002) was used to segment the left and right hippocampus of each subject into separate binary image volume masks. These masks were used as seed regions for generation of deterministic tractography in DTI Query v1.1 (Sherbondy et al., 2005) and probabilistic tractography in FSL v4.1.4 (Behrens et al., 2003).

Hippocampal volume-tractography analyses

From the segmented binary hippocampal masks, indices of inter-hemispheric hippocampal volume differences were computed as:

$$\frac{NDhipp\_vol - Dhipp\_vol}{NDhipp\_vol + Dhipp\_vol}$$

where *NDhipp_vol* and *Dhipp_vol* are volumes (cubic mm) of the healthy and sclerotic hippocampus, respectively.

DW-MRI tractography can be used to model fiber systems by generating streamlines, or pathways, through white matter. In deterministic tractography, these pathways follow the direction, or eigenvector, corresponding to the principle eigenvalue in voxels that meet a threshold level (here, 0.2) of fractional anisotropy. In probabilistic tractography, multiple pathways may be generated through a single voxel if a probability density function suggests the existence of multiple fiber orientations. Thus, both deterministic and probabilistic algorithms generate numbers of pathway. The more pathways, the greater the volume of brain tissue is represented. While the absolute numbers of pathways may differ between algorithms due to differences in tractography parameters, like seed spacing and step size, the number of pathways seeded or connecting a given area can be considered as a measure of fiber volume or density. In this study, the inter-hemispheric difference in pathway number was considered as a measure of connectivity density.

Indices of inter-hemispheric hippocampal connectivity density differences were computed as:

$$\frac{NDhipp\_ntracts - Dhipp\_ntracts}{NDhipp\_ntracts + Dhipp\_ntracts}$$

where *NDhipp_ntracts* and *Dhipp_ntracts* are numbers of tractography pathways from the healthy and sclerotic hippocampal seed masks, respectively. No constraints were made on the crossing of fiber pathways from each hippocampus into the contralateral hemisphere.

Once pathways are generated by tractography algorithms, it is possible to quantify properties of tissue microstructure in all voxels represented by the pathways. In this study, the fractional anisotropy values in voxels occupied by pathways are integrated to derive a single mean FA value for different sets of hippocampal pathways. It is possible that, in one hippocampus a large number of pathways may connect to the rest of the brain but they may pass through voxels having a lower mean FA than the mean FA of another set of pathways connecting the contralateral hippocampus. Since FA is a measure of white matter integrity that summarizes the directional dependence of water diffusion, the term connectivity strength is used to distinguish it from connectivity density.

Indices of inter-hemispheric hippocampal connectivity strength differences were computed as:

$$\frac{NDhipp\_fa - Dhipp\_fa}{NDhipp\_fa + Dhipp\_fa}$$

where *NDhipp_fa* and *Dhipp_fa* are mean fractional anisotropy of voxels representing the tractography pathways from the healthy and sclerotic hippocampal seed masks, respectively.

For control subjects, the three indices above were computed by substituting left and right hippocampus into these equations, as neither hippocampus is diseased.

Results

To illustrate how the inter-hemispheric volume and connectivity indices were computed, the patient hippocampal volumes and tractography pathway and FA values are listed in Table 1.

To illustrate the spatial distribution of pathways connecting each hippocampus, a group map is shown (Fig. 1) for the 8 right hemisphere patients computed by probabilistic tractography.

Hippocampal volumes

As expected from other morphometric studies of hippocampal sclerosis, in our patient sample the volume of the hippocampus in the diseased hemisphere was on average 21% smaller ($Dhipp\_vol = 3146\,mm^3$ vs. $NDhipp\_vol = 3972\,mm^3$, $p < 0.008$). There was no significant volume difference between the left and right hippocampus of the healthy controls (4300 vs. 4264, $p = 0.40$).

Relationship between hippocampal volumes and tractography density

As expected, in patients a significant positive correlation was found between inter-hemispheric hippocampal volume and connectivity density (Fig. 2a, $r = 0.71$, $p = 0.02$ for deterministic tractography and $r = 0.98$, $p < 0.0001$ for probabilistic tractography) suggesting that tractography pathway numbers decrease with decreases in hippocampal volume. In healthy controls, no correlation was found between hippocampal volume and connectivity density using deterministic tractography ($r = 0.25$, $p = 0.48$), but a significant positive relationship was found using probabilistic tractography ($r = 0.99$, $p < 0.0001$). The positive relationship found in both patients and in controls with probabilistic tractography suggests that the number of pathways that this algorithm generates is sensitive to even very small changes in hippocampal volume.

Relationship between hippocampal volumes and tractography strength

Surprisingly, in patients a significant inverse relationship was found between inter-hemispheric hippocampal volume and connectivity strength (Fig. 2b, $r = -0.67$, $p = 0.03$ for deterministic tractography and $r = -0.71$, $p = 0.02$ for probabilistic tractography). This result indicates a different directional dependence of water diffusion as a function of the volume of the sclerotic and non-sclerotic hippocampi. In healthy controls, no significant correlation was found between hippocampal volume and connectivity strength using either deterministic ($r = -0.40$, $p = 0.24$) or probabilistic ($r = -0.04$, $p = 0.92$) methods, indicating, on average, equal levels of directionally dependent water diffusion between the hippocampi of healthy controls.

Similarity between volume—density and volume—strength relationships

To ensure that the differences in the relationships between connectivity and strength with respect to hippocampal volume in the patient group were similar between tractography algorithms, the results were correlated. The deterministic and probabilistic methods produced a pattern of very similar results ($r = 0.75$, $p = 0.01$ for density and $r = 0.69$, $p = 0.03$ for strength).

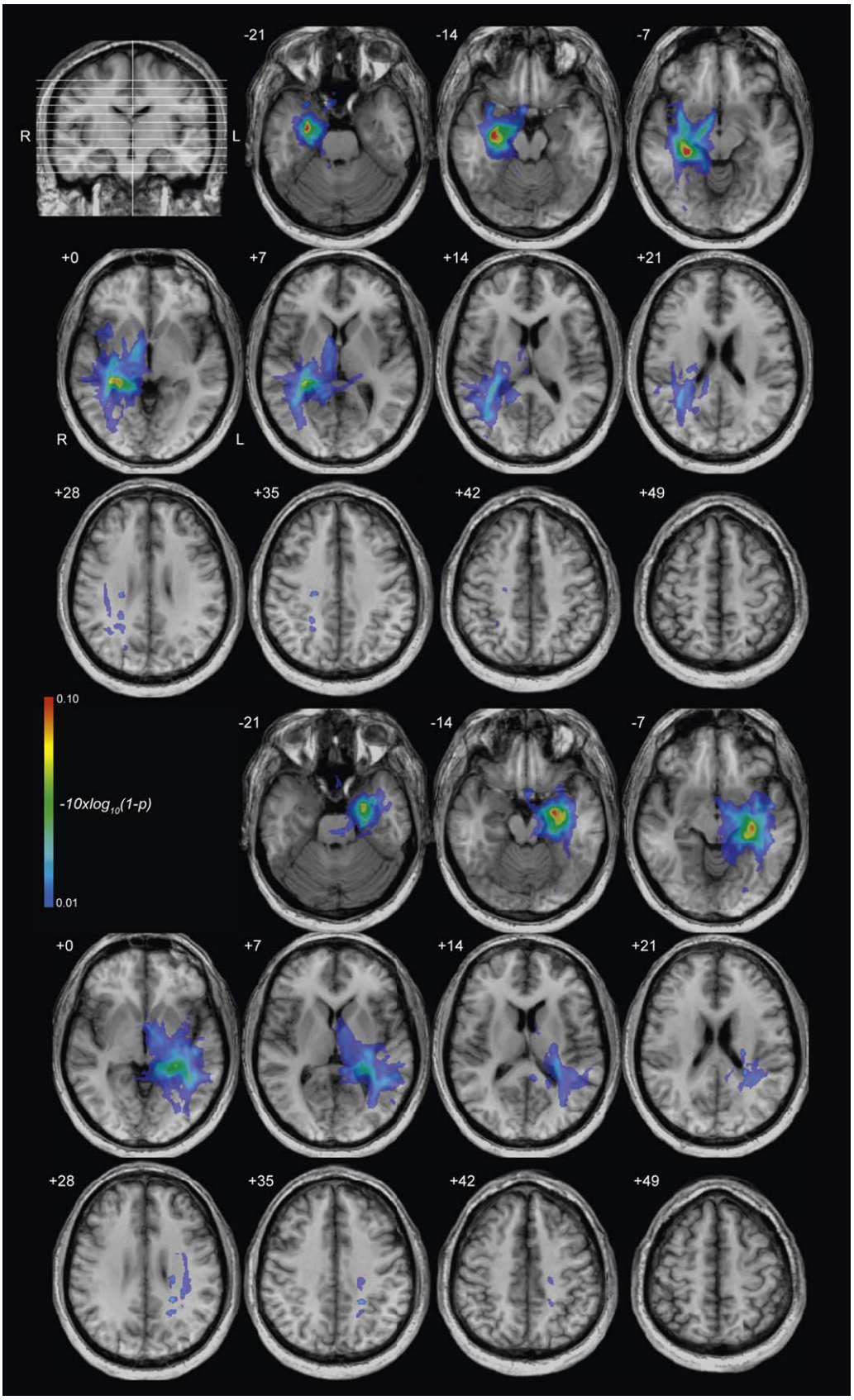
Discussion

We used a validated automated method for segmenting the hippocampus from T1-weighted MRIs, and found smaller hippocampal volumes accompanying HS in TLE patients. Our average effect size for volume reduction was remarkably

Table 1 Patient hippocampal volumes, pathway numbers, and pathway strength (FA) values.

Patient	Volume (mm3)		# Paths (Det.)		# Paths ($\times 10^3$) (Prob.)		Mean FA (Det.)		Mean FA (Prob.)	
	NDHipp	*DHipp*	*NDHipp*	*DHipp*	*NDHipp*	*DHipp*	*NDHipp*	*DHipp*	*NDHipp*	*DHipp*
1	3577	2689	1252	923	3075	2165	0.4122	0.4144	0.2970	0.3009
2	3861	2554	1490	1412	3250	2115	0.3809	0.3955	0.2855	0.2854
3	3717	2756	3859	3021	3220	2345	0.4122	0.4293	0.2835	0.2861
4	4150	2863	2294	1596	3610	2485	0.3863	0.3937	0.2712	0.2712
5	5194	4591	1622	1613	4530	3945	0.4105	0.4003	0.2504	0.2597
6	3199	2934	983	1455	2700	2605	0.3995	0.4051	0.2550	0.2708
7	4144	3185	1818	1709	3415	2775	0.4317	0.4303	0.2929	0.2898
8	4630	3046	2229	1636	3890	2540	0.3923	0.3947	0.2652	0.2813
9	3292	1867	1579	768	2990	1720	0.3582	0.3966	0.2724	0.2832
10	3956	4982	1543	1621	3390	4210	0.4179	0.4093	0.2885	0.2726
Mean	3972.00	3146.70	1866.90	1575.40	3407.00	2690.50	0.4002	0.4069	0.2762	0.2801
Stdev	603.79	939.43	805.09	599.44	514.19	790.96	0.0213	0.0138	0.0159	0.0117

DHipp: sclerotic hippocampus; *NDHipp*: healthy hippocampus; Det.: deterministic; Prob.: probabilistic.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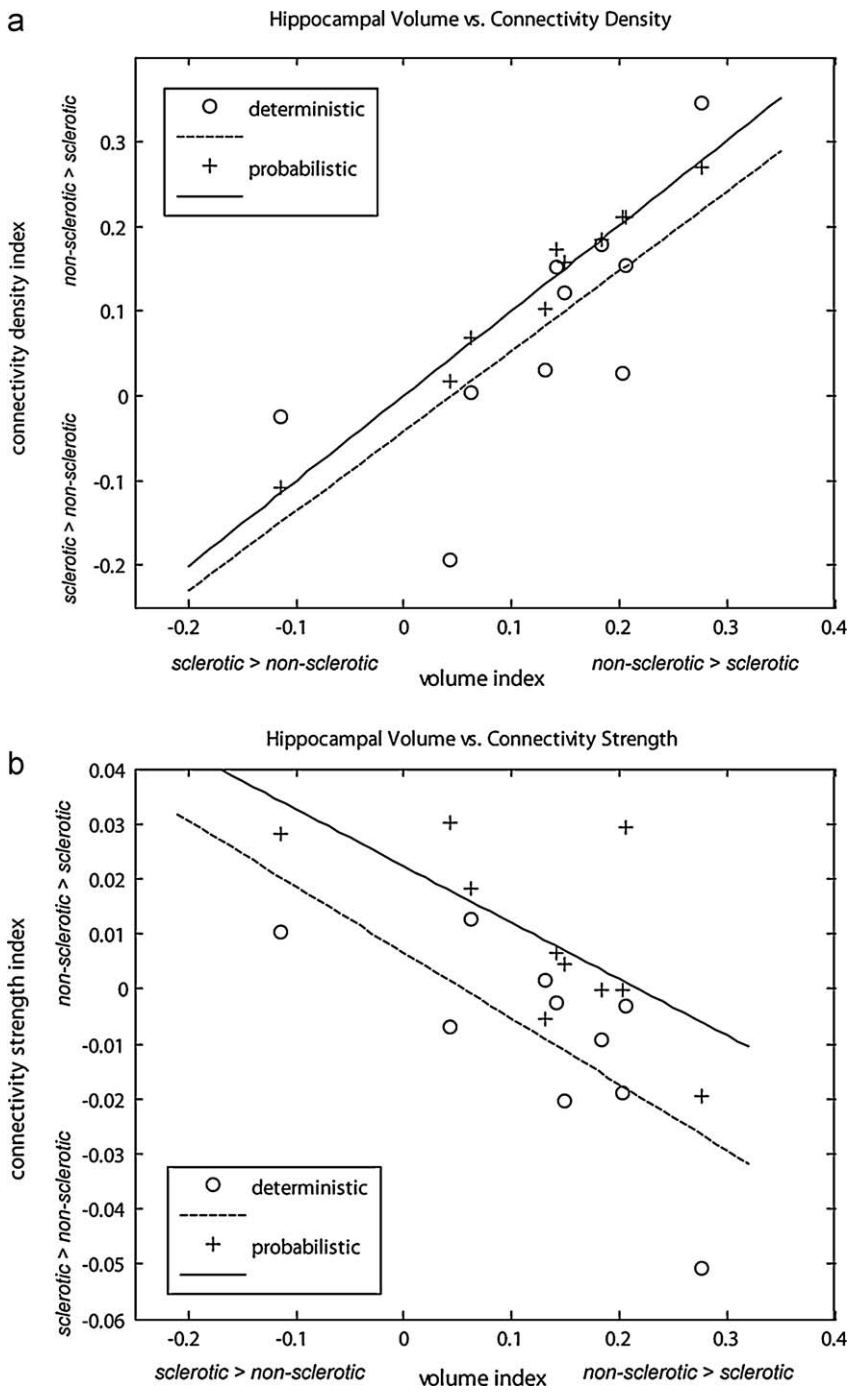

Figure 2 Dissociation between hippocampal connectivity density and strength. Connectivity density (# of reconstructed tractography pathways) is positively related to hippocampal volume (a), but connectivity strength (fractional anisotropy of reconstructed pathways) is inversely correlated with hippocampal volume (b). Similar relationships are found using deterministic and probabilistic methods.

Figure 1 Probabilistic tractography seeded from left and right hippocampus. An average ($n = 8$ right HS patients) probabilistic tractography map is shown in color overlaid on an average brain constructed from the patients' T1-weighted MRIs and transformed into MNI space. Planes are spaced 8 mm apart at levels indicated by white lines on the coronal planes in the upper left corner inset. The top three rows are tractography results from the right (sclerotic) hippocampus and the bottom three rows are tractography results seeded from the left (non-sclerotic hippocampus). The volume of tissue covered by tractography seeded from the sclerotic hippocampus is 8.6% smaller than the volume of tissue covered by tractography seeded from the non-sclerotic hippocampus.

similar (21% vs. 20%) to that found by another group using the same automated segmentation method on HS patients (Pardoe et al., 2009). When tractography pathways were seeded from both hippocampi in the patients and controls, we found evidence that density as measured by the number of tractography pathways was positively correlated with hippocampal volume. In the patients, this means that the volume reduction accompanying HS corresponds to a reduction in the number of white matter fiber tracts connecting the sclerotic hippocampus to the rest of the brain. This is not unexpected, as there is likely to be more axons occupying a greater volume of tissue in the non-sclerotic hippocampus. However, when we analyzed the strength of connectivity as measured by the mean fractional anisotropy of voxels representing the reconstructed tractography pathways, we found an inverse relationship between hippocampal volume and connectivity strength in the patients, but not in the matched control subjects. In the patients, this means that relative volume reductions in the sclerotic hippocampus are not accompanied by reductions in pathway strength, but by relative increases in pathway strength. In our sample, this is shown by changes in the connectivity strength index (− for sclerotic > non-sclerotic, + for non-sclerotic > sclerotic) which scale with the degree of hippocampal atrophy. The finding of relative increased connectivity strength as a function of hippocampal volume reduction was obtained with both deterministic and probabilistic methods, which suggests that this is a robust phenomenon. This novel result carries some implications for the pathophysiology of seizure spread and raises an important question regarding the neurobiology underlying this difference in connectivity: in TLE patients with HS, does the smaller atrophic hippocampus, which is shown here to have fewer pathways connecting it to the rest of the brain, actually facilitate the spread of seizures by way of its stronger relative connectivity?

Diffusion imaging has been used previously in HS patients to identify hippocampal and extrahippocampal changes in white matter microstructure (Assaf et al., 2003; Bonilha et al., 2010; Concha et al., 2009; Focke et al., 2008; Knake et al., 2009; Liacu et al., 2010; Powell et al., 2007; Salmenpera et al., 2006; Thivard et al., 2005), and three main findings have emerged. First, using region-of-interest and voxel-based statistical parametric mapping methods, increased diffusivity and decreased fractional anisotropy have been noted in the sclerotic compared to non-sclerotic hippocampus (Assaf et al., 2003; Concha et al., 2009; Focke et al., 2008; Knake et al., 2009; Liacu et al., 2010; Salmenpera et al., 2006). Second, there is some evidence that connectivity is decreased in the hemisphere ipslilateral to HS in the small subset of studies that have employed tractography methods (Powell et al., 2007; Bonilha et al., 2010). Finally, abnormal diffusion patterns are not confined to the hippocampus (Focke et al., 2008; Gross et al., 2006), but are widespread throughout the affected hemisphere and correlate with gray matter atrophy in other structures (Bonilha et al., 2010).

In the DW-MRI studies reviewed above, a pattern of hippocampal deafferentation or disconnection is suggested by findings of lower regional white matter integrity (FA) and decreased connectivity as measured by fewer tractography pathways. The unique methodological approach employed by the present study relates hippocampal volume changes accompanying HS to inter-hemispheric connectivity density as measured by tractography pathway numbers and connectivity strength as measured by integrating FA within the tissue represented by the tractography pathways. By computing connectivity density and strength separately, we demonstrate the novel finding of a volume reduction in the sclerotic hippocampus that is accompanied by a relative decrease in connectivity density, but a relative increase in connectivity strength. Our finding of reduced connectivity density confirms previously reported results suggesting hippocampal deafferentation accompanying HS, but our finding of increased connectivity strength also suggests that the fewer number of connections have relatively augmented directional dependence of water diffusion. Additional investigations will be required to understand whether the dissociation of density and strength is caused by the long-term effect of seizure activity or whether it facilitates seizure spread during the early development of epilepsy.

This study is the first to explore relationships among connectivity density, connectivity strength and hippocampal volume by application of deterministic and probabilistic diffusion tractography methods. Our finding of dissociation between connectivity density and strength in epilepsy patients is novel; it is significant in that it may be related to the neurobiological changes that underlie seizure propagation in the atrophic hippocampus. Intense synaptic reorganization has been shown to take place in the epileptic hippocampus, which includes axonal sprouting (Magloczky, 2010) in addition to cell loss. We propose that the present finding of a relative increase in fractional anisotropy within a smaller relative number of tractography pathways may be an important macroscopic correlate of the synaptic reorganization that takes place within hippocampal and extrahippocampal brain structures in epilepsy.

We conclude that morphological differences in the hippocampus of epilepsy patients with HS include not only volume differences, but also distinct differences in connectivity density and strength as measured by deterministic and probabilistic DW-MR tractography.

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Conflcts of interest

None declared.

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