Septal nuclei enlargement in human temporal lobe epilepsy without mesial temporal sclerosis

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ABSTRACT

Objective: To measure the volume of basal forebrain septal nuclei in patients with temporal lobe epilepsy (TLE) as compared to patients with extratemporal epilepsy and controls. In animal models of TLE, septal lesions facilitate epileptogenesis, while septal stimulation is antiepileptic.

Method: Subjects were recruited from two sites and consisted of patients with pharmacoresistant focal epilepsy (20 with TLE and mesial temporal sclerosis [MTS]; 24 with TLE without MTS, 23 with extratemporal epilepsy) and 114 controls. Septal volume was measured using high resolution MRI in association with newly-developed probabilistic septal nuclei maps. Septal volume was compared between subject groups while controlling for relevant factors.

Results: Patients with TLE without MTS had significantly larger septal nuclei than patients with extratemporal epilepsy and controls. This was not true for patients with MTS. These results are interpreted with reference to prior studies demonstrating expansion of the septo-hippocampal cholinergic system in animal models of TLE and human TLE surgical specimens.

Conclusion: Septal nuclei are enlarged in TLE patients without MTS. Further investigation of septal nuclei and antiepileptic septo-hippocampal neurocircuitry could be relevant to development of new therapeutic interventions such as septal stimulation for refractory TLE.

INTRODUCTION

Human septal nuclei, located in the basal forebrain, consist of the medial septum and diagonal band of Broca. Septal nuclei provide the main cholinergic input to the hippocampus via the fimbria/fornix[1] and are critical for hippocampal theta oscillations that mediate learning and memory.[2, 3] In animal models of temporal lobe epilepsy (TLE), medial septal lesions facilitate epileptogenesis, and chemical or electrical septal stimulation can stop and prevent seizures.[4-8] To our knowledge, no studies have examined septal nuclei structure in human TLE.

We used MRI in association with probabilistic maps of human septal nuclei[9] to measure septal volume in patients with TLE either with or without mesial temporal sclerosis (MTS), patients with partial epilepsy not involving the hippocampus (extratemporal epilepsy; ETE) and healthy controls. Based on prior human MRI studies showing atrophy of hippocampi and connected limbic regions in TLE including the fornix,[10, 11] we initially hypothesized that septal volume would be reduced in TLE. Contrary to this hypothesis, we found septal enlargement in TLE without MTS.

METHODS

Subjects

All subjects provided informed consent to participate in this IRB-approved study. Patients with epilepsy were recruited from either the NYU or UCSD Epilepsy Center. See **Table 1** for subject information. All patients had medically intractable seizures and were being considered for epilepsy surgery. Seizure focus lateralization/localization was made by experienced epileptologists based on clinical criteria including neuroimaging, video-EEG, and intracranial EEG (icEEG) when available. TLE patients were categorized as having either MTS (TLE-MTS) or not based on standard criteria.[12] Patients without MTS are referred to as TLE-NL, though 5/24 did have subtle mesial temporal abnormalities that did not qualify as MTS (1 with subtle parahippocampal gray-white blurring, 1 with slight hippocampal increased FLAIR signal but no atrophy, 3 with mild atrophy but no signal change). Patients with TLE due to tumors or other non-MTS lesions were not included. ETE patients had either normal MRI (n=15), incidental non-cortical abnormalities (n=2), or subtle focal cortical dysplasia (FCD) characterized by signal change or gray-white blurring at the seizure focus (n=4). Patients with grossly abnormal MRI which could interfere with image processing, serious medical/neurological disease other than epilepsy, or serious psychiatric disease such as psychosis were not included. Controls were recruited through online advertisement and denied medical, neurologic or psychiatric illness.

Table 1. Subject characteristics.					
group	**n**	**sex**	**age (std)**	**epilepsy duration (std)**	**lateralization/localization**
NYU TLE-NL	18	10F	36.0 (10.2)	17.7 (15.3)	9 left, 8 right, 1 bilateral; icEEG performed in 10/18
UCSD TLE-NL	6	3F	35.5 (9.6)	21.3 (15.4)	4 right, 2 left
NYU TLE-MTS	6	4F	36.8 (8.4)	20.4 (13.4)	3 left, 3 right; icEEG performed in 1/6
UCSD TLE-MTS	14	9F	37.2 (13.4)	27.2 (11.8)	4 right, 10 left
ETE (NYU only)	23	13F	28.5 (11.0)	20.3 (11.4)	10 left, 8 right, 5 bilateral/uncertain; 15 frontal/central, 8 parietal/occipital; icEEG performed in 12/23
NYU controls	89	43F	34.5 (12.5)	n/a	n/a
UCSD controls	25	13F	34.1 (10.2)	n/a	n/a
Subject groups did not differ significantly by age ($F[6,174]=1.22$, $p=.30$), epilepsy duration ($F[4,61]=1.08$, $p=.37$) or sex (Pearson $\chi^2[6, n=181]=2.12$, $p=.91$).					

MRI scanning

High resolution research MRI was performed at either the NYU Center for Brain Imaging on a 3T Siemens Allegra head-only scanner or at the UCSD Radiology Imaging Laboratory on a GE 1.5T EXCITE scanner (NYU: TE=3.25ms, TR=2530ms, TI=1.1ms, FOV=256mm, voxel size=1x1x1.33mm; UCSD: TE=3.8ms, TR=10.7ms, TI=1000 ms, FOV=240mm, voxel size=1.25x1.25x1.2mm) .

Measurement of septal volume

Using Statistical Parametric Mapping (SPM8, Wellcome Trust Center for Neuroimaging) individual scans were normalized to the MNI152 T1-template using a 12-parameter affine transformation and partitioned into gray and white matter using a unified segmentation approach. Gray and white matter maps were registered to the segmented MNI152 T1-template using the DARTEL toolbox, an efficient large deformation diffeomorphic framework which provides information about voxel-level local expansion and contraction necessary to deform the image to match the template.[13] The DARTEL flow fields derived from this registration were applied to a binary mask of the septal nuclei (generated as described below). To warp the septal maps, which were in MNI template space, back to each individual subject's native space, we applied

the inverse DARTEL flow field. We then calculated the volume of each subject's septal mask in mm^3. We have used this method previously.[14]

The binary septal nuclei masks we used were based on probabilistic maps of magnocellular (presumably cholinergic) basal forebrain cell groups generated using digital images of cell-stained histological sections from 10 human postmortem brains which were reconstructed in 3D using the MRI scans of the fixed brain as a shape reference, then spatially normalized to the single-subject T1-weighted MNI reference brain, as described in detail elsewhere.[9] The masks included all voxels showing ≥10% probability of being part of the medial septal nucleus or the nucleus of the diagonal band of Broca, corresponding to Mesulem's Ch1-Ch2 cell group.[1, 9]

To allow correction for baseline differences in intracranial volume across subjects, total intracranial volume (TICV) was calculated using Freesurfer 5.0 (http://surfer.nmr.mgh.harvard.edu).

Statistical Analysis

Statistical analyses were performed using SPSS. NYU and UCSD datasets were combined and were also analyzed separately. One-way analysis of covariance (ANCOVA) was conducted with independent variable = subject group (TLE, ETE [for NYU dataset only], and controls); covariates = age, TICV and, when appropriate, site; and dependent variable = total septal volume. To assess an effect of MTS on septal volume, analyses were also performed with TLE patients split into separate groups based on the presence or absence of MTS (TLE-MTS, TLE-NLI). For patient groups, partial correlation analyses were performed to assess a possible effect of epilepsy duration on septal volume while controlling for age, TICV and, when appropriate, site.

Because septal nuclei are located adjacent to and in between lateral ventricles, and ventricular enlargement (which can occur in TLE[15]) could potentially distort septal volume measurement, we used Freesurfer to calculate total lateral ventricle volume (TLVV), and performed all analyses both with and without TLVV as an additional covariate.

All results were considered significant at $p<.05$.

RESULTS

Mean septal volume are presented in **Table 2. Figure 1** shows an example of septal nuclei segmentation.

TLE vs Controls

There was a highly significant effect of group indicating that TLE patients had larger septal nuclei than controls ($F[1,153]=12.9$, $p<.0005$) when controlling for age, TICV and site. These results were similar when TLVV was included as an additional covariate ($p=.002$), and when NYU and UCSD datasets were analyzed separately ($p=.017$ and $p=.037$, respectively.)

TLE-NL vs TLE-MTS vs Controls

When the TLE group was divided into those with and without MTS, septal volume still differed significantly by group ($F[2,152]=7.432$, $p=.001$), and pairwise comparisons indicated that only TLE-NL patients had significantly larger septal volumes than controls ($p<.0005$). TLE-MTS patients did not differ from TLE-NL patients ($p=.172$) nor from controls ($p=.120$). Results when TLVV was included as an additional covariate were similar ($p<.0005$), except a trend for greater septal volume in TLE-NL as compared to TLE-MTS patients became apparent ($p=.079$). Note that for this comparison, NYU and UCSD datasets were not analyzed separately because there were only 6 NYU TLE-MTS patients and only 6 UCSD TLE-NL patients.

TLE-NL vs TLE-MTS vs ETE vs Controls

In the NYU dataset, which included ETE patients, septal volume differed significantly by group ($F[3,130]=3.808$, $p=.012$), and pairwise comparisons indicated that TLE-NL patients had significantly larger septal volumes than controls ($p=.002$) and than ETE patients ($p=.004$). There was a weak trend for TLE-NL patients to have larger septal volume than TLE-MTS patients ($p=.093$). TLE-MTS patients did not differ from controls ($p=.910$) nor from ETE patients ($p=.778$). Results were similar when TLVV was included as an additional covariate in analysis ($p<.0005$).

Correlation with epilepsy duration

There was no correlation between septal volume and epilepsy duration when controlling for age, TICV and site in TLE patients overall ($r=.088$, $p=.584$) nor in TLE-MTS nor TLE-NL subgroups ($r=.142$, $p=.587$; $r=.111$, $p=.632$; respectively). These results did not change when TLVV was included as an additional covariate, nor when NYU and UCSD datasets were analyzed separately.

There was a weak trend for greater septal volume with epilepsy duration in ETE patients ($r=.38$, $p=.095$), and this trend became stronger when TLVV was included as an additional covariate ($r=.45$, $p=.056$).

Table 2. Mean Septal Volumes

group	N	septal volume (mm3)	Std. Dev.
NYU TLE-NL	18	1762.78	253.64
UCSD TLE-NL	6	1786.67	329.86
NYU TLE-MTS	6	1681.00	165.67
UCSD TLE-MTS	14	1614.00	164.85
ETE (NYU only)	23	1660.30	176.54
NYU control	89	1734.89	218.32
UCSD control	25	1734.24	197.01

DISCUSSION

Results demonstrate that patients with TLE have larger septal nuclei than controls. Septal enlargement in TLE is limited to patients without MTS; septal volume in TLE patients with MTS does not differ from that in controls. The specificity of our results – that septal nuclei are enlarged only in TLE and not in extratemporal epilepsy – is in accord with the known anatomic specificity of septal nuclei connections with hippocampi.[1, 2]

Our results contrast with multiple prior MRI studies (none of which assessed septal nuclei) showing atrophy of hippocampi and interconnected limbic structures in TLE both with and without MTS, considered to reflect neuronal loss in the seizure focus and regions of propagated seizure activity.[10, 11] Hippocampal seizures propagate to septal nuclei,[4, 16] so how can we explain our finding of septal enlargement rather than atrophy in TLE patients without MTS?

A potential explanation comes from animal studies demonstrating that septal cholinergic neurons are resistant to seizure-induced neuronal loss[8, 17] and may actually *enlarge* in TLE via a mechanism dependent upon Nerve Growth Factor (NGF) released from hippocampus and transported to septal nuclei retrogradely along the fornix.[18, 19] NGF is an essential growth factor for septal cholinergic neurons, and its production is highly upregulated by hippocampal seizures.[20] While neuronal cell body enlargement does not correspond directly to regional enlargement detectable by MRI, we believe this unusual propensity of septal cholinergic neurons to survive and enlarge in an animal model of TLE provides a relevant model for understanding our MRI finding of septal enlargement in human TLE.

This translational framework also offers an explanation for septal enlargement limited to TLE patients without MTS: severe hippocampal cell loss might preclude production of sufficient NGF in response to seizures to cause septal enlargement. In support of this idea, septal atrophy occurs following hippocampal excitotoxic ablation[21] or septo-hippocampal disconnection.[19]

In the context of these prior animal studies, as well as studies of human tissue obtained at temporal lobectomy indicating *increased* septal cholinergic innervation of hippocampus in TLE,[22-24] we suggest our finding of septal enlargement in human TLE without MTS represents MRI-detectable evidence of neuroplasticity/augmentation of the septal-hippocampal system in TLE.

Understanding the mechanism, consequences and cellular basis of septal enlargement in human TLE without MTS will require additional studies. Histological examination of septal tissue obtained at autopsy and/or noninvasive imaging tests such as neuroreceptor Positron Emission Tomography using cholinergic and other ligands can clarify the cellular basis of MRI-detected septal enlargement. Longitudinal studies will be important to ascertain whether septal enlargement is cause, consequence or both of TLE. In this respect, our results showing no effect of epilepsy duration on septal volume in TLE patients suggests that this process may occur early in TLE, and our finding of a trend towards larger septal nuclei with longer duration of extratemporal epilepsy could perhaps indicate progression of ETE to involve the hippocampus and the septo-hippocampal system over time.[25]

Given the role of septo-hippocampal projections neurons in dampening hippocampal hyperexcitability and stopping seizures,[4-8] a better understanding of septal nuclei in TLE could open up new antiepileptic therapeutic avenues such septal stimulation. Septal stimulation can abort seizures in animals,[6] and stimulation of septal outflow tract in human is safe, and may improve memory.[26]

Methodologic Issues

The main findings of our study are based on a combined NYU-UCSD dataset. While combining data across different MR scanners can be problematic, it has become increasingly common in the era of large, multisite neuroimaging studies,[27] and is generally considered appropriate so long as both patients and controls are scanned at each site, and site is included as a covariate in analysis,[28] as we have done. In addition, we analyzed each dataset separately, and results were concordant.

Ventricle Volume as confound

Septal nuclei location in between and adjacent to lateral ventricles could distort their measurement, due to ventricular enlargement and/or edge effects.[29] By explicitly measuring lateral ventricle volume, we were able to control for this effect. It will be important for future studies, especially those focused on disorders associated with marked ventricular enlargement such as dementia, to also measure and account for ventricle volume when assessing septal volume.

In conclusion, we have demonstrated that septal nuclei are enlarged in patients with TLE without MTS. This initial MRI finding in humans can be understood with reference to animal and human studies demonstrating preservation and/or expansion of the septal-hippocampal cholinergic systems in TLE. Further investigation of this understudied antiepileptic neural circuit could be relevant to development of new therapeutic interventions for refractory TLE.

FIGURE LEGEND

Figure 1. Coronal view of septal nuclei in (**A**) a patient with TLE and (**B**) a control.

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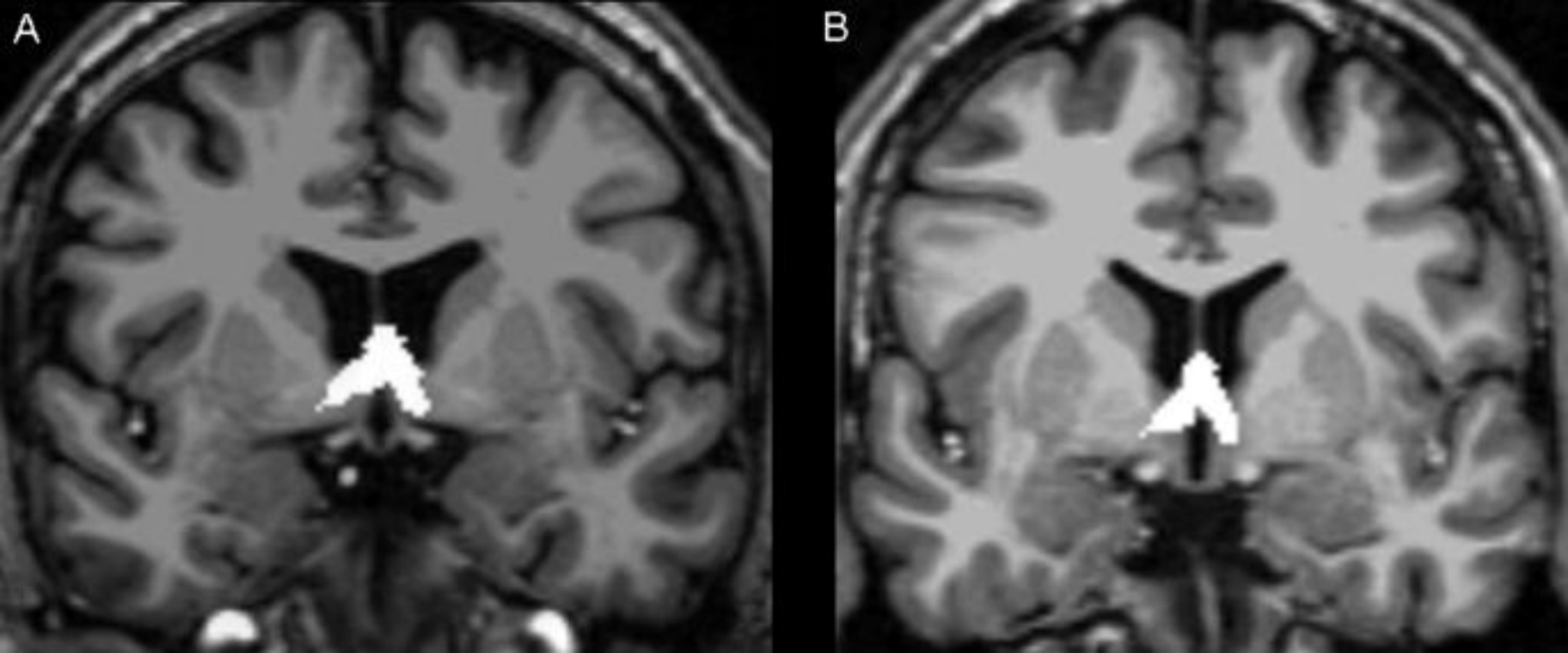
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