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Granule cell dispersion is associated with memory impairment in right mesial temporal lobe epilepsy

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ABSTRACT

Purpose: We analyzed the association of granule cell dispersion (GCD) with memory performance, clinical data and surgical outcome in a series of patients with mesial temporal lobe epilepsy (MTLE) and mesial temporal sclerosis (MTS).
Method: Hippocampal specimens from 54 patients with MTLE (27 patients with right MTLE and 27 with left MTLE) and unilateral MTS, who were separated into GCD and no-GCD groups and thirteen controls were studied. Quantitative neuropathological evaluation was performed using hippocampal sections stained with NeuN. Patients' neuropsychological measures, clinical data, type of MTS and surgical outcome were reviewed.
Results: GCD occurred in 28 (51.9%) patients. No correlation between GCD and MTS pattern, clinical data or surgical outcome was found. The presence of GCD was correlated with worse visuospatial memory performance in right MTLE, but not with memory performance in left MTLE.
Conclusion: GCD may be related to memory impairment in right MTLE-MTS patients. However, the role of GCD in memory function is not precisely defined.

1. Introduction

Mesial temporal sclerosis (MTS) is the most common type of lesion abnormality observed in mesial temporal lobe epilepsy (MTLE).[1] Other than segmental neuronal loss within the hippocampal subfields, different patterns of pathology are often described in the dentate gyrus (DG), including granule cell dispersion (GCD), bilaminar DG, granule cell loss, and ectopic granule cells in the molecular layer of the DG.[2]

GCD is a common phenomenon that occurs in approximately 50% of all MTS specimens.[3] The criteria for determining dispersion vary from subjective qualitative impressions of the width of the molecular layer to objective quantitative measures of width.[4,5] Little is known about the pathogenic mechanisms and clinical significance of GCD. Seizures that occur early in the maturing hippocampus[6,7] and developmental abnormalities have been hypothesized to cause GCD.[8] Previous studies have failed to correlate GCD with seizure outcome following surgery for MTLE.[2,9,10] The association between GCD and clinical data also remains controversial.[6,7,11]

The hippocampus plays a crucial role in learning and memory. Explicit episodic memory is strongly associated with mesial temporal structures[12,13] and is the most striking cognitive deficit in MTLE patients.[14,15] Verbal memory deficits are more commonly associated with the left mesial epileptogenic zone, whereas nonverbal memory impairments are more frequently associated with non-dominant MTLE.[16,17] Seizure frequency, duration of epilepsy, the use of antiepileptic drugs (AEDs), and interictal epileptiform discharges have been implicated in memory dysfunction observed in MTLE patients.[18–20] Histopathological investigation of surgical specimens may help to understand memory impairment in these patients.[2,21]

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Human studies and animal models have correlated the degree of cell loss in the hippocampus and DG with memory decline.[22,23] Neurogenesis of DG cells may play a role in behavior and the acquisition of new memories in rodents.[24,25] Similar mechanisms are likely to occur in humans.[21]

The influence of GCD in the formation of memory remains unclear. To address this issue, we studied the occurrence of GCD in the hippocampi of refractory MTLE-MTS patients and investigated the relationship between this abnormality and memory performance, clinical characteristics, MTS patterns, and surgical outcome.

2. Materials and methods

2.1. Subjects

Fifty-four patients (27 men), who were identified as right-handed using the Handedness Inventory[26], with medically refractory MTLE and unilateral MTS (27 right MTS and 27 left MTS) visualized via MRI were included in the present study. These patients are part of a previously published series of studies.[27] The mean age was 38.5 ± 10.48 years for the right MTLE patient group and 36.7 ± 10.84 years for the left MTLE group. Right and left MTLE patients were divided into GCD and no-GCD groups. Diagnosis of MTLE was established according to previously reported clinical and electrographic characteristics.[28] Patients were defined as medically refractory if seizure was not controlled using at least two first-line AEDs, in mono or polytherapy up to toxic levels.

This retrospective study is based on review of previously collected tissue samples. This study was approved by the institutional Ethics Committee, and all participants gave informed consent.

Clinical features were reviewed using a specific protocol developed for this study that focused on six variables. Presence and age of the initial precipitant insult (IPI) were assessed as defined by Mathern et al.[29] The patient age at onset of habitual seizures, which was defined as the age at which habitual and recurrent seizures developed, was recorded. Similarly, the duration of epilepsy, which was defined as the interval between the age at onset of habitual seizures and the time of surgery, was noted. We also examined the average frequency of preoperative complex partial seizures (CPS) per month, as well as the approximate number of generalized tonic–clonic seizures (GTCS) throughout life, which was categorized as $>$ or $<$20.

All patients completed an extensive preoperative evaluation, which included high-resolution MRI and prolonged noninvasive video-EEG recording. Visual inspection of the MRI images revealed that all patients had clear evidence of MTS, which includes varying degrees of hippocampal sclerosis (e.g., atrophy, increased T2-and decreased T1-weighted signal, and disrupted internal structure of the hippocampus) as well as atrophy and signal alteration of the amygdala and temporal pole. Patients with unilateral MTS that was associated with structural abnormalities other than brain atrophy were excluded.

Surgical resection was performed when this evaluation yielded evidence of MTLE with MTS. This surgery consisted of resection of 3.5 cm of the temporal neocortex from the temporal pole of the dominant hemisphere and 4.5 cm from the non-dominant hemisphere. This surgical resection yielded 3.5 cm of hippocampus and approximately two-thirds of the amygdala. All patients were followed for seizure control according to Engel's classification[30] for at least six months following the surgery.

2.2. Neuropsychological measures

A standard clinical measure of intelligence was used (estimated full scale IQ from the Wechsler Adult Intelligence Scale-Revised [WAIS-R]).[31] The Logical Memory I and II subtests[32] (immediate and delayed recall) were used to assess verbal memory, and the Visual Reproduction I and II subtests[32] (immediate and delayed recall) were used to assess visual memory. In addition, the Rey–Osterrieth Complex Figure Test[33] (immediate and delayed recall) was used to assess visuospatial memory, and the Rey Auditory Verbal Learning Test (RAVLT)[33] was used to asses verbal learning.

2.3. Tissue preparation

The hippocampi of all patients were collected from the archives of neuropathology from the years 2005 to 2011. Samples containing less than three hippocampal subfields or lacking layers CA1 and/or CA4 were excluded.

The hippocampus was dissected into five-mm-thick slices along the anterior–posterior axis, fixed overnight in a 4% formalin solution and then processed in liquid paraffin. A single block of tissue corresponding to the mid-hippocampal body was selected from each case for histopathological examination.[34] Blocks were cut to 7 μm (Leica, Germany), expanded in hot water, mounted on slides coated with saline and air-dried overnight in an incubator at 56 °C. Two slides from each case were deparaffinized in xylol and a descending alcohol concentration. One slide was stained with hematoxylin and eosin (HE) for pathological diagnosis of MTS, and the other slide was submitted to an automated staining apparatus (Autostainer Link 48, Dako, USA) for NeuN immunohistochemistry (Chemicon, Temecula, USA, dilution 1:1000, pre-treated in PT Link equipment, Dako, USA) and hematoxylin counterstaining. Two independent observers (AJ and RN) completed quantitative and qualitative analyses of all tissue samples, which included slides stained with NeuN and HE.

Specimens collected at autopsy from the hippocampi of thirteen neurologically healthy individuals, without a history of epilepsy, served as controls (mean age 57.6 ± 14.34 years). Postmortem tissue samples were stained with HE and NeuN. As reported in previous studies[5,9] there was no difference in accuracy between controls' HE- and NeuN-stained sections.

2.4. Morphologic analysis and neuronal cell counts

Microscopic images were obtained using an E600 microscope equipped with a Moticam 2300 camera (Nikon, Japan). Measurements of granule cell layer (GCL) width and neuronal cell counts were performed with ImageJ software (National Institutes of Health, NIH, USA). Immunohistochemically stained neuronal cell bodies were visualized on a computer screen (Motic Images Plus software).

2.4.1. Granule cell dispersion

GCD was considered present when three conditions were observed concurrently: (a) straight portions of the GCL were greater than 120 μm in width; (b) granule cells were not in close opposition to each other; and (c) the boundary between the molecular layer and the GCL was no longer clearly defined.[4]

Sections stained with NeuN were used to calculate the average width of the GCL in each surgical case. The distance from the inner border of the GCL to the outer border of the most distal granule cell was determined in the internal as well as in the external limbs and mid portion of the DG. Eight regions of maximal GCD along the GCL were measured[9] to calculate the mean and standard deviation of each case. Curved regions were not considered.

2.4.2. Neuronal cell counts

All tissue samples collected from MTLE patients and controls were analyzed according to Blumcke et al.[5] Hippocampal subfields were identified using a 2× objective lens; four randomly selected

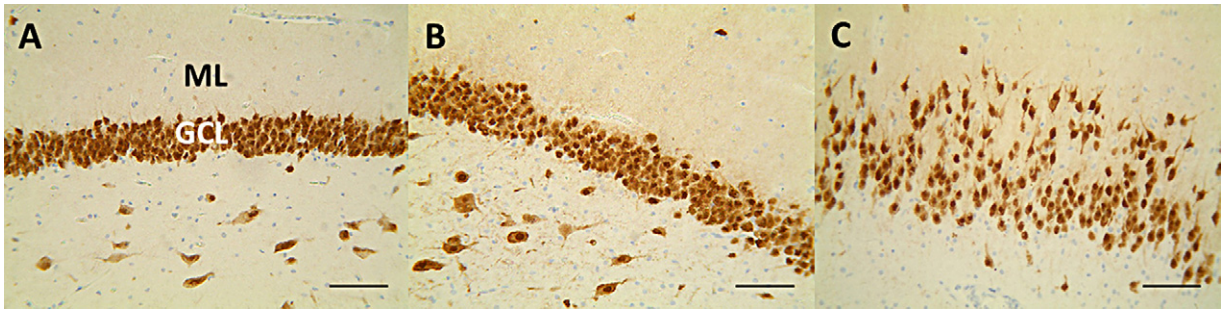

Fig. 1. Histopathological findings in the dentate gyrus of controls and MTLE patients (NeuN-stained sections). (A) Control; (B) No-GCD: granule cells are densely packed and have a distinct border with the ML; (C) GCD: granule cells are dispersed and spread into the ML; width of GCL exceeded 120 μm. Scale bars, 100 μm. GCD: granule cell dispersion, GCL: granule cell layer, ML: molecular layer, MTLE: mesial temporal lobe epilepsy.

regions of 0.0625 mm^2 in areas were outlined in each sector (CA1–CA4). All NeuN-positive neurons, despite any differences in size or morphology, were counted in the selected fields under 20× magnification. For each subfield, neuronal cell density was calculated in neurons × $10^{-4}/\mu m^2$.

2.5. Statistical analysis

Neuronal cell counts from each hippocampal subfield were transformed into *z*-scores. *z*-Score is the number of standard deviations from the mean expected value, which in this case is the mean neuronal density in the controls' hippocampal subfields. *z*-Scores for neuronal density were calculated according to the following formula: z = (observed density score − mean control density score)/standard deviation of the control density score. Neuronal cell counts greater than two standard deviations away from the mean were considered abnormal; only negative *z*-scores were relevant. Five different patterns of HS were defined by the following criteria: no MTS (*z*-score <2 in all hippocampal subfields); MTS type 1a (*z*-score <2 in CA2 but >2 in all other sectors); MTS type 1b (*z*-score >2 in all subfields); MTS type 2 (*z*-score >2 in CA1 and <2 in CA4); and MTS type 3 (*z*-score >2 in CA4).[9]

Statistical analysis was completed with SPSS 10.0 for Windows, Version 10.01. Student's *t*-test was used to compare clinical data between different groups. The results (means ± SD) obtained from all of the memory tests administered to patients with right and left MTLE with MTS, including those with and without GCD, were compared and transformed into *z*-scores. For this comparison, a one-way analysis of variance (ANOVA) was performed. When appropriate, the post hoc Bonferroni procedure was performed; P values <0.05 were considered statistically significant.

To investigate whether memory impairment could be secondary to variables other than the presence of GCD, such as duration of epilepsy, age at surgery, monthly seizure frequency, MTS type and postoperative outcome, we performed an analysis of covariance (ANCOVA) in right and left MTLE-MTS groups.

3. Results

GCD was observed in 28 cases (51.9%): 14 in right MTLE and 14 in left MTLE (Fig. 1). Clinical variables, including age at onset, presence and type of IPI, duration of the latency period, duration of epilepsy and age at surgery were similar between the GCD and no-GCD groups in both right MTLE and left MTLE. No statistically significant difference was found between right MTLE and left MTLE patients in terms of their years of education and IQ (Table 1). MTS pattern was not associated with GCD in MTLE-MTS patients (Table 2). Monthly seizure frequency and estimated lifetime GTCS were similar in both groups.

Postoperative outcomes were similar in right MTLE-MTS and left MTLE-MTS patients as well as in the GCD and no-GCD groups. Among the right MTLE patients, 85.7% with GCD and 76.9% without GCD were seizure-free after the surgery. Of the left MTLE patients, 78.6% with GCD and 69.2% without GCD were seizure-free following the surgery.

In right MTLE patients, the GCD group obtained significantly lower scores on the immediate recall portion of the Rey–Osterrieth Complex Figure Test. This finding may suggest that visuospatial memory is more greatly affected by GCD in right MTLE. However, in the left MTLE-MTS group, the presence of GCD was not associated with memory performance. Table 3 illustrates the mean adjusted *z*-scores for these groups.

An analysis of covariance was used to control relevant variables that might influence memory impairment. The results of the ANCOVA showed that these variables had no impact on memory performance; thus, GCD appears to be the only variable affecting memory performance in this sample of MTLE patients.

4. Discussion

Houser first described GCD in patients with TLE.[8] Hippocampal specimens collected from these patients lacked a distinct boundary between the granule cell and molecular layers, and numerous

Table 1
Clinical data (mean ± SD) of MTLE patients in GCD and no-GCD groups.

	Right MTLE patients			Left MTLE patients		
	No-GCD (N = 13)	GCD (N = 14)		No-GCD (N = 13)	GCD (N = 14)	
Sex (M/F)	7/6	4/10	P = 0.252	6/7	10/4	P = 0.252
Education (years)	9.3 ± 4.61	8.3 ± 3.47	P = 0.519	6.8 ± 4.80	6.9 ± 4.22	P = 0.960
IQ	80.6 ± 9.20	83.4 ± 11.72	P = 0.496	84.4 ± 11.51	81.1 ± 13.85	P = 0.507
IPI Percentage (N)	38.5% (5)	50.0% (7)	P = 0.704	53.8% (7)	64.3% (9)	P = 0.704
FS Percentage (N)	60.0% (3)	28.6% (2)	P = 0.667	57.1% (4)	44.4% (4)	P = 1.000
Latency (years)	13.8 ± 5.72	10.4 ± 5.06	P = 0.305	16.0 ± 8.14	13.4 ± 6.77	P = 0.504
Epilepsy onset (years)	16.7 ± 8.40	17.6 ± 9.34	P = 0.783	14.5 ± 8.65	11.9 ± 9.12	P = 0.461
Epilepsy duration (years)	17.3 ± 8.63	24.5 ± 12.80	P = 0.102	24.7 ± 10.77	23.2 ± 15.72	P = 0.780
Age at surgery (years)	34.6 ± 11.33	42.1 ± 8.48	P = 0.060	38.7 ± 8.31	34.9 ± 12.79	P = 0.368

GCD: granule cell dispersion; M: male; F: female; IPI: initial precipitant insult; FS: febrile seizure; N: number of patients; SD: standard deviation.

Table 2
Distribution of MTS patterns in GCD and no-GCD MTLE patients groups.

	Right MTLE patients		Left MTLE patients	
	No-GCD ($N=13$)	GCD ($N=14$)	No-GCD ($N=13$)	GCD ($N=14$)
No MTS	7.7% (1)	0.0% (0)	15.4% (2)	0.0% (0)
MTS type 1a	38.5% (5)	21.4% (3)	23.1% (3)	42.9% (6)
MTS type 1b	53.8% (7)	78.6% (11)	46.2% (6)	50.0% (7)
MTS type 2	0.0% (0)	0.0% (0)	15.4% (2)	7.1% (1)
	$P=0.298$		$P=0.448$	

GCD: granule cell dispersion; *N*: number of patients.

granule cell somata extended into the molecular layer. Further studies demonstrated that GCD is not exclusive to patients with HS. In a postmortem study, GCD was observed in patients who had widespread cortical malformations without hippocampal cell loss, suggesting a neurodevelopmental disorder.[35]

In our study of pure unilateral MTLE-MTS, GCD occurred in 51.9% of all cases, which is consistent with data previously reported in the literature.[5,9] The association of GCD with clinical characteristics such as history of IPI and seizure frequency has been discussed previously.[6,7,11] We found no correlation between these clinical variables and GCD. Considering that the presence of GCD was not correlated with clinical factors, GCD may be more closely linked to the pathological mechanism of MTS rather than being a manifestation of severe TLE.[11]

Our data are consistent with previous studies that suggest that there is no correlation between GCD and seizure outcome.[5,9,10] Classic patterns of MTS expression were most frequently observed in our sample of MTLE patients, including both the GCD and no-GCD group. This finding is consistent with previous reports[5]; however, there was no statistically significant difference in MTS pattern expression observed in these groups of patients.

Quantitative histological analysis of the tissue resected from the hippocampal subfields and DG has been associated with pre- and post-operative memory deficits.[36,37] In a human electrophysiological study, Grunwald et al.[38] reported that limbic P300 (a memory related component) recorded from electrodes placed at a intrahippocampal depth correlated significantly with neuronal density of the DG, but not with pyramidal cell density in hippocampal subfields CA1 through CA4. Animal studies confirmed this finding.[23,39] Blumcke et al.[2] collected data from 26 patients; the authors observed significantly better memory performance in patients without dentate granule cell loss or dispersion. The same group[21] found that neuronal cell loss within the internal limb of the dentate gyrus, a developmentally distinct subregion of the hippocampal formation known to generate new neurons throughout life, was a highly accurate predictor of the ability to learn and recall memories.

Although previous studies have proposed a relationship between granule cell abnormality and memory function during intracarotid amobarbital procedure (IAP),[2,21] IAP was not used in our study of pure unilateral MTLE-MTS we tested memory function using a number of neuropsychological assessments, which are commonly administered in other epilepsy centers. Despite its diagnostic value, IAP is an invasive procedure; complications have been reported in up to 11.6% of patients.[40] Although IAP has often been cited as the gold standard in assessment of language lateralization, it has not been established as a predictor of postoperative memory decline. Rather, postoperative memory decline can generally be measured using noninvasive methods. Baxendale et al.[41] suggested that it is inappropriate to conduct an invasive procedure, such as IAP, solely to gain prognostic data regarding postoperative memory decline.

We did not find a significant relationship between GCD and memory performance in left MTLE-MTS patients. However, GCD was associated with visuospatial memory impairment in right MTLE-MTS patients on the immediate, but not the delayed recall portion of the Rey–Osterrieth Complex Figure Test. Retention of visuospatial learning processes may not be as greatly affected possibly because memory of novel stimuli is widely represented in the entire brain.[42] Instruments used to assess nonverbal memory functions are considered to be not as sensitive as those used to assess verbal memory.[43] IAP results have been most predictive of verbal memory changes in patients with dominant temporal lobe epilepsy. However, IAP results have not fared as well as a predictor of nonverbal memory changes after resection of areas of the non-dominant hemisphere.[44,45] Previous studies have also failed to confirm that the extent of right hippocampal pathology is related to performance on test of non-verbal memory.[22,46] The findings of this study may increase knowledge and understanding of the clinical role of GCD in memory impairment in MTLE patients.

The DG generates neurons throughout life[47] and may play a role in the acquisition of new memories.[24] Animal studies have described the functional integration of newly generated neurons and its impact on memory acquisition.[24,48] These data indicate that new granule cells are not only affected by the formation of hippocampal–dependent memory but also participate in it. These granule cells may also increase with efforts to learn and recall memories. Coras et al.[49] studied neural stem cells from 23 surgically sampled human hippocampi and assessed the neurogenic potential of DG cells. Patients with high proliferation capacity stem cells performed normally on assessments of memory prior to epilepsy surgery, whereas those with low proliferation capacity showed severe learning and memory impairments. The authors concluded that encoding new memories was related to the regenerative capacity of the hippocampus in the human brain. In

Table 3
Visual and verbal memory test results (*z*-score ± SD) obtained from right and left MTLE patients in GCD and no-GCD groups.

	Right MTLE		Left MTLE		Bonferroni post hoc analysis (*P* value)	
	No-GCD ($N=13$)	GCD ($N=14$)	No-GCD ($N=13$)	GCD ($N=14$)	Between R-MTLE GCD and no-GCD	Between L-MTLE GCD and no-GCD
Visual Reproduction I	−0.43 (0.847)	−0.84 (0.714)	−0.58 (0.881)	−0.81 (1.084)	NS	NS
Visual Reproduction II	−0.72 (0.799)	−1.37 (0.916)	−0.72 (0.950)	−0.94 (1.228)	NS	NS
Rey Complex Figure II	−1.49 (1.293)	−2.84 (0.912)	−1.31 (1.290)	−1.13 (1.538)	0.05	NS
Rey Complex Figure III	−0.92 (0.944)	−1.52 (0.544)	−0.94 (0.699)	−0.68 (0.991)	NS	NS
Logical Memory I	−0.80 (1.054)	−0.76 (0.772)	−0.99 (0.968)	−1.07 (0.853)	NS	NS
Logical Memory II	−1.08 (0.869)	−0.94 (0.772)	−1.18 (0.838)	−1.29 (0.816)	NS	NS
RAVLT – Total	−0.62 (0.841)	−1.14 (1.385)	−0.57 (1.302)	−1.27 (1.117)	NS	NS
RAVLT – 30 min	−0.76 (0.511)	−1.09 (1.219)	−1.04 (1.135)	−1.61 (1.282)	NS	NS
RAVLT – Recognition	0.22 (0.590)	−0.68 (1.598)	0.05 (0.813)	−0.31 (1.445)	NS	NS

GCD: granule cell dispersion; *N*: number of patients; NS: not significant. Values presented in mean (SD).

our study, neurogenesis was not examined; studies involving newly generated neurons and memory function are therefore highly encouraged.

It should be noted that the hippocampus contralateral to the resected side in MTLE patients may play a role in memory functions that would otherwise be attributed to the resected hippocampus. This process, know as memory plasticity, may be present in early onset cases in particular and may have impacted the results of our analyses, thus representing a limitation of our study.

In conclusion, the occurrence of GCD was associated with visuospatial memory deficit in right MTLE patients. However, the presence of GCD was not associated with memory performance in left MTLE patients. The present findings emphasize the importance of performing a histopathological evaluation as part of the epilepsy surgery protocol as well as the contribution of histopathology to understanding memory performance in TLE patients. However, the role of GCD in memory is not yet precisely defined.

Conflict of interest

The authors have no conflicts of interest to declare.

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