Available online at www.sciencedirect.com
SciVerse ScienceDirect
www.elsevier.com/locate/brainres

Research Report

Altered hippocampal myelinated fiber integrity in a lithium-pilocarpine model of temporal lobe epilepsy: A histopathological and stereological investigation

Yuanzhen Ye[a], Jiajia Xiong[a], Jun Hu[a], Min Kong[a], Li Cheng[a], Hengsheng Chen[a], Tingsong Li[a,b,*], Li Jiang[a,b,*]

[a]Lab of Pediatric Neurology, Ministry of Education Key Laboratory of Child Development and Disorders, Key Laboratory of Pediatrics in Chongqing, Chongqing International Science and Technology Cooperation Center for Child Development and Disorders, Children's Hospital of Chongqing Medical University, Chongqing, PR China
[b]Department of Neurology, Children's Hospital of Chongqing Medical University, 136# Zhongshan 2 Road, Chongqing 400014, PR China

ARTICLE INFO

Article history:
Accepted 16 May 2013
Available online 28 May 2013

Keywords:
Temporal lobe epilepsy
Demyelination
Stereology
Hippocampus
Myelinated fiber

ABSTRACT

The damage of white matter, primarily myelinated fibers, in the central nervous system (CNS) of temporal lobe epilepsy (TLE) patients has been recently reported. However, limited data exist addressing the types of changes that occur to myelinated fibers inside the hippocampus as a result of TLE. The current study was designed to examine this issue in a lithium-pilocarpine rat model. Investigated by electroencephalography (EEG), Gallyas silver staining, immunohistochemistry, western blotting, transmission electron microscopy, and stereological methods, the results showed that hippocampal myelinated fibers of the epilepsy group were degenerated with significantly less myelin basic protein (MBP) expression relative to those of control group rats. Stereological analysis revealed that the total volumes of hippocampal formation, myelinated fibers, and myelin sheaths in the hippocampus of epilepsy group rats were decreased by 20.43%, 49.16%, and 52.60%, respectively. In addition, epilepsy group rats showed significantly greater mean diameters of myelinated fibers and axons, whereas the mean thickness of myelin sheaths was less, especially for small axons with diameters from 0.1 to 0.8 μm, compared to control group rats. Finally, the total length of the myelinated fibers in the hippocampus of epilepsy group rats was significantly decreased by 56.92%, compared to that of the control group, with the decreased length most prominent for myelinated fibers with diameters from 0.4 to 0.8 μm. This study is the first to provide experimental evidence that the integrity of hippocampal

Abbreviations: TLE, temporal lobe epilepsy; CNS, central nervous system; MBP, myelin basic protein; DTI, diffusion tensor imaging; pilo, pilocarpine; LiCl, lithium chloride; EEG, electroencephalography; PBS, phosphate-buffered saline; DAB, diaminobenzidine; BSA, bovine serum albumin; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; TEM, transmission electron microscope; IUR, uniform and random; MRI, magnetic resonance imaging; BBB, blood–brain barrier

*Corresponding authors at: Department of Neurology, Children's Hospital of Chongqing Medical University, 136# Zhongshan 2 Road, Chongqing 400014, PR China. Fax: +86 23 6362 2754.
E-mail addresses: neurojiang@gmail.com, 170127625@qq.com.(L. Jiang)

http://dx.doi.org/10.1016/j.brainres.2013.05.026

myelinated fibers is negatively affected by inducing epileptic seizures with pilocarpine, which may contribute to the abnormal propagation of epileptic discharge.

1. Introduction

Temporal lobe epilepsy (TLE) is the most common form of focal epilepsy and is especially refractory to drug treatment in adults (Engel, 1996). Although epilepsy is historically considered to be a gray matter disease with hippocampal sclerosis that consists of gliosis and neuronal loss, the major associated pathology of TLE, recent diffusion tensor imaging (DTI)

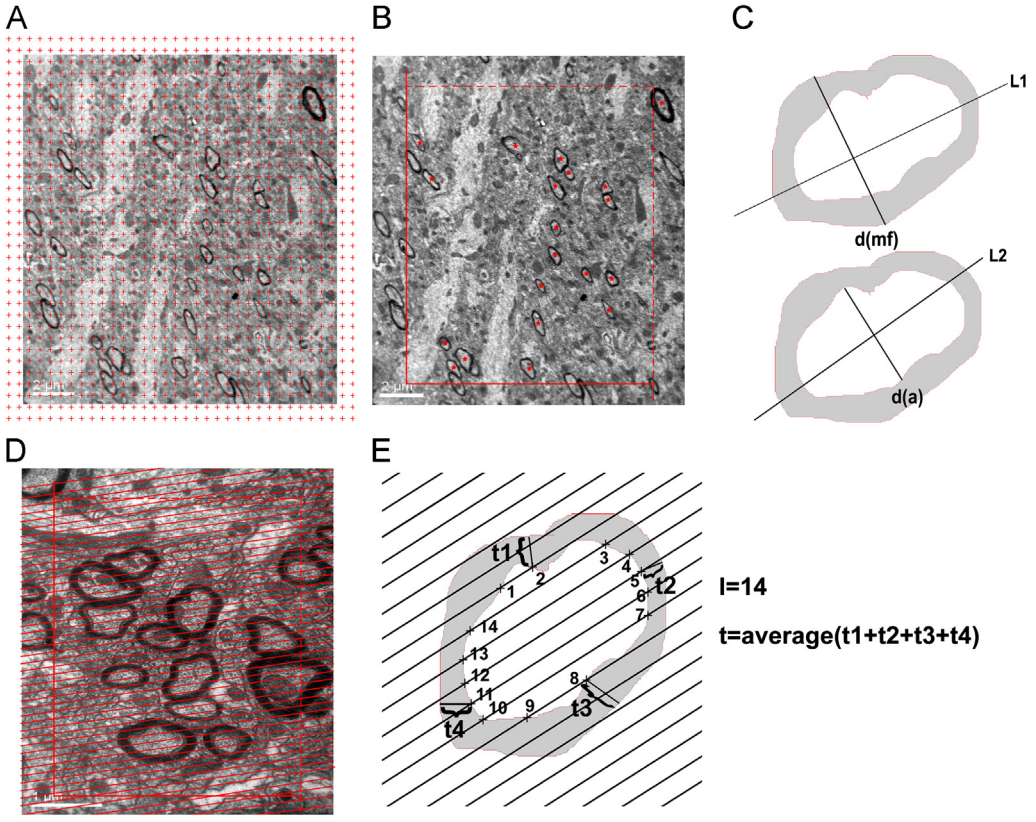

Fig. 1 – Stereological methods. (A) The number of points hitting the hippocampal formation, the myelinated fibers, and the myelin sheath were counted, respectively. (B) Principle of unbiased counting frame: all myelinated fiber profiles inside the counting frame, provided they do not touch or intersect the full-drawn lines, were considered for counting. ★ indicates the myelinated fibers counted. In this illustrated photograph, the number of counted profiles = 20. (C) All myelinated fiber profiles completely inside the counting frame or partly inside it but not touching or intersecting the full-drawn lines were used for diameter measurement. Upper: The diameters of the myelinated fibers (d(mf)) sampled with the unbiased counting frame were estimated by measuring the myelinated fiber profile diameter perpendicular to the longest axis of the myelinated fiber (L1). Lower: The axonal diameter (d(a)) was estimated by measuring the axonal profile diameter perpendicular to the longest axis of the axon (L2). (D) A grid of parallel two-dimensional lines were overlaid randomly on the captured photograph. All myelinated fiber profiles completely inside the counting frame or partly inside it but not touching or intersecting the fully-drawn lines were sampled. (E) The number of intersections between the profile boundary and the test parallel lines, *I*, was counted (in this illustrated figure, there are 14 intersections between the profile boundary and the test lines). The orthogonal myelin sheath thickness, *t*, for each myelinated fiber was estimated as an average of four measurements of four points described as follows. The points of intersection were numbered consecutively, the first point was chosen randomly in the first *I*/4 interval, the second point was *I*/4+point 1, the third point was *I*/4+point 2, and the fourth point was *I*/4+point 3. In this illustrated figure, the shortest distances from the inner boundary to the outer boundary at four positions were measured, which are indicated by *t*1, *t*2, *t*3, and *t*4. The mean thickness of the myelin sheath equals the mean value of *t*1, *t*2, *t*3, and *t*4. (In the given example, *I* = 14, so the first position should be chosen at random from numbers 1–3. In this illustrated figure, intersection 2 was randomly chosen as the first position. The second position was *I*/4+position 1 (intersection 5). The third position was *I*/4+position 2 (intersection 8), and the fourth position was *I*/4+position 3 (intersection 11).) Eight rats were used for the control group and seven were used for the pilo group.