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Proliferative hippocampal activity in a group of patients with Rasmussen's encephalitis: Neuronal, glial, and BDNF tissue expression correlations

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ABSTRACT

Rasmussen's encephalitis (RE) is a rare and devastating unilateral inflammatory brain disease that causes severe and intractable partial epilepsy. It has been shown that epilepsy and subsequent inflammation have deleterious influence on hippocampal cell survival and neurogenesis, but this still has not been systematically explored in human tissue. In this study, we investigated the correlation between inflammation and epilepsy as well as the rates of hippocampal gliogenesis and neurogenesis in a pediatric group of six patients with RE and six control cases. The dentate gyrus (DG) samples were obtained from patients who underwent surgery for intractable RE. Sections were processed for immunohistochemistry using antibodies against sex determining region Y-box 2 (Sox2), nestin, human protein encoded by MKI67 gen (Ki67), and brain-derived neurotrophic factor (BDNF). There was an increase in the number of Ki67-positive granule cells in the DG of patients with RE in comparison with the autopsy control group, but no statistical difference for Sox2-positive cells was observed between these groups. Nestin immunolabeling was less intense in the RE group while BDNF expression was increased. Neurons that were BDNF-positive were found in DG from patients with RE but not in the control group. In patients with RE, few nestin-positive cells in DG were also positive for BDNF, unlike in controls which showed no colocalization for these two markers. These results suggest a proliferation activity in the DG subfield of patients with RE, and also future studies are necessary to address the role of new cells in the hippocampus of patients with RE.

1. Introduction

Rasmussen's encephalitis (RE) is an inflammatory disease frequently observed in children aged 3 to 11 years old. Its main symptom is the frequent occurrence of seizures refractory to most known anticonvulsant drugs, and its most significant underlying substrate is the presence of a severe inflammatory process limited to one cerebral hemisphere that may progressively culminate in hemiparesis or contralateral hemiplegia to the affected hemisphere. The strong inflammatory component involves microglia activation, numerous microglial nodules, astrocytosis, and cytotoxic T-cell recruitment culminating in neuronal loss [1].

The etiology of RE is not fully established, but it has been long believed that viral infection in early childhood plays a role on the pathophysiology of the disease [2]. Nevertheless, a final demonstration of a viral agent for RE has not been accomplished yet [3–12].

Neurogenesis is a persistent brain ability to generate new neurons from neural stem/progenitor cells (NSCs), as demonstrated by the remarkable work by Joseph Altman and Gopal Das in the 1960s, which first suggested that the adult brain has the ability to generate these new brain cells [13]. Similar suggestions were made by Michael Kaplan in the 1970s [14] for mammalian brains and by Fernando Nottebohm in the 1980s for avian brains [15]; however, adult neurogenesis was accepted by the scientific community only in the early 1990s [16–18].

Currently, it is well-established that there are two main neurogenic niches in the adult brain: the subventricular zone (SVZ) [19] on the wall of the lateral ventricles and subgranular zone (SGZ) on the hippocampal dentate gyrus (DG). In rodents, B cells (an astrocyte-like cell) generate neuroblasts that migrate to the olfactory bulb in which they are integrated in functional adult circuits [20]. In the hippocampus, progenitor cells generate neuroblasts, which migrate to the granular cell layer, then becoming new granular cells [21]. The meaning of adult neurogenesis is yet to be known, but it has been suggested that the new cells are important to the maintenance and repair of interneurons in the OB, modulation and refinement of adult circuits in the

Abbreviations: RE, Rasmussen's encephalitis; NSCs, neural stem/progenitor cells; Sox2, sex determining region Y-box 2; Ki67, human protein encoded by MKI67 gen; NeuN, neuronal nuclei protein.

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Table 1
Patients' clinical history. Hippocampus subfield availability was the factor used to include the subjects. RE = patients with Rasmussen's encephalitis; F = female; M = male; NA = datum not available.

Patients	Clinical history				
	Gender	Age at epilepsy onset	Duration of epilepsy	Age at surgery	Side of resection
RE1	F	2	1	3	Left
RE2	M	6	2	8	Left
RE3	F	6	9	15	Left
RE4	F	NA	NA	NA	NA
RE5	F	2	1	3	NA
RE6	F	8	1	9	Left

hippocampus, as well as to some specific kind of hippocampal-dependent memory [22].

Different factors such as an enriched environment [21], learning [23], stress [24,25], physical exercise [26–28], seizures [29], and inflammation [30,31] influence adult neurogenesis. Also, seizures may play a controversial time-dependent role on increasing adult neurogenesis [32,33] or diminishing it after long-term seizure episodes [34].

Although further understanding on increased neurogenesis after seizures is needed, it may be some sort of effort made by the injured brain to replace cells lost after seizure activity. Nevertheless, it has been shown that the newly born cells do not survive for a long time after a neurological insult, which jeopardizes integration into adult circuits [30]. Moreover, these newly born cells probably form anomalous circuits, which can increase the occurrence of seizures instead of improving the patient's overall clinical condition [35]. On the other hand, the inflammation effects on the developing brain and neurogenesis rates are extensively researched, and the inflammatory process is accepted as essential for tissue maintenance and repair; however, exacerbated and long sustained inflammation is extremely harmful not only for normal brain maintenance but also for rates of physiological neurogenesis [36]. Together with the growing interest in neurogenesis, the factors that influence this phenomenon also instigate intense curiosity. Brain-derived neurotrophic factor (BDNF) is a neurotrophin that has been associated with the occurrence of neurogenesis, but many authors associate this expression with an increase of brain injury in epilepsy [37]. Rasmussen's encephalitis is a good candidate to understand this correlation because this disease presents both of these processes together. Furthermore, there is no well-established information about such correlation between RE and neurogenesis, neither in humans nor in an animal model of epilepsy. Therefore, the present study aimed at evaluating neuronal cell loss, astrocytosis, neurogenesis rates, and neurotrophin BDNF pattern in hippocampal tissue from patients with RE in comparison with human autopsy control cases.

2. Material and methods

2.1. Human hippocampal tissue

Hippocampal specimens were obtained from 6 patients who underwent epilepsy surgery at the Erlangen Epilepsy Centre or São Paulo Hospital, Federal University of São Paulo, in which drug-resistant RE was diagnosed by preoperative evaluation. Presurgical epilepsy monitoring included interictal and ictal video-electroencephalography (video-EEG) monitoring, using 32- to 64-channel EEG, as well as magnetic resonance imaging (MRI) (1.5 Tesla Sonata Siemens, Munich, Germany) and neuropsychological evaluation. Intracarotid amobarbital procedure (WADA test), positron emission tomography (PET), magnetic encephalography, and intraoperative electrocorticography were applied when necessary to characterize the epileptogenic zone [38]. Mean duration of epilepsy was 2.8 years, ranging from 1 to 9 years. The average age at the surgery was 7.6 years, ranging from 3 to 15 years old (see Table 1). Informed and written consent was given by all patients included in our study for additional scientific investigations approved by the local ethics committee of the University of Erlangen. All procedures were conducted in accordance with the Declaration of Helsinki (1964).

The control group consisted of autopsy cases (age range from 5 to 27 years old) from the University Hospital Erlangen, of which the cause of death was not related to the nervous system.

2.2. Histopathological examination

Each surgical hippocampus specimen was dissected into 5-mm thick slices along the anterior–posterior axis. Tissue from the mid-hippocampal body was fixed overnight in 10% formalin and routinely processed into liquid paraffin. All specimens were cut at 4 μm on a rotation microtome (Microm, Germany) and stained with hematoxylin and eosin. Hippocampal pyramidal neurons and granule cells of the DG were detected using immunohistochemistry for neuronal nuclei protein (NeuN) (Millipore, 1:1000) in an automated staining apparatus (Ventana, Strasbourg, France). Microwave pretreatment was applied for paraffin-embedded tissue labeling.

2.3. Immunohistochemistry and immunofluorescence

The following antibodies were used: NeuN, anti-glial fibrillary acid protein (GFAP), nestin, BDNF, sex determining region Y-box 2 (Sox2), human protein encoded by MKI67 gen (Ki67), and β-III tubulin. Immunocytochemistry of fixed human hippocampus tissue and quantitative microscopic evaluation were performed as described earlier [39]. Primary antibodies were used at the following dilutions: mouse anti-NeuN (1:100, Chemicon-Millipore®), goat anti-GFAP (1:100, DAKO®),

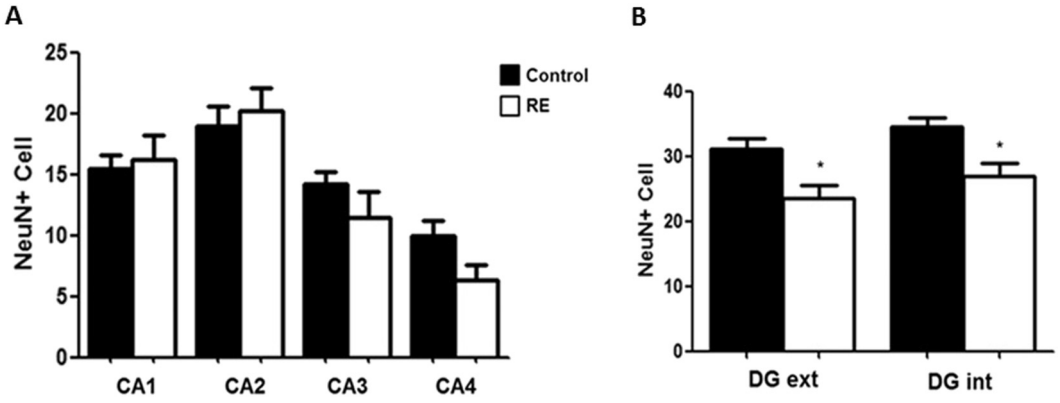

Fig. 1. Hippocampal neuronal cell counting: Mean number of neurons in hippocampal (A) subfields and the dentate gyrus (B) in control (black bars) and RE cases (white bars). (*$p > 0.05$ — ANOVA, Bonferroni's multiple comparison posttest, no significant difference).