Characterization of Intracranial Pressure Behavior in Chronic Epileptic Animals: A Preliminary Study

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Abstract Intracranial pressure (ICP) is a major neurological parameter in animals and humans. ICP is a function of the relationship between the contents of the cranium (brain parenchyma, cerebrospinal fluid, and blood) and the volume of the skull. Increased ICP can cause serious physiological effects or even death in patients who do not quickly receive proper care, which includes ICP monitoring. Epilepsies are a set of central nervous system disorders resulting from abnormal and excessive neuronal discharges, usually associated with hypersynchronism and/or hyperexcitability. Temporal lobe epilepsy (TLE) is one of the most common forms of epilepsy and is also refractory to medication. ICP characteristics of subjects with epilepsy have not been elucidated because there are few studies associating these two important neurological factors. In this work, an invasive (ICPi) and the new minimally invasive (ICPmi) methods were used to evaluate ICP features in rats with chronic epilepsy, induced by the experimental model of pilocarpine, capable of generating the main features of human TLE in these animals.

Keywords Intracranial pressure • Epilepsy • ICP • ICP monitoring • Pilocarpine

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Introduction

Intracranial pressure (ICP) is the pressure inside the skull. It is derived from cerebral blood and cerebrospinal fluid circulatory dynamics and can be affected during the course of many diseases of the central nervous system (CNS) [6]. The vascular component (cerebral blood) is difficult to express quantitatively, and it is probably derived from the pulsation of the cerebral blood volume detected and adjusted by nonlinear mechanisms of cerebral blood volume regulation.

In most organs of the human body, the environmental pressure for blood perfusion is either low or coupled to atmospheric pressure. The environmental pressure for CNS differs in this respect as the brain is surrounded and protected by a rigid skull. An increase in intracranial pressure may impede blood flow and result in ischemia [6].

More generally, multiple variables such as arterial pressure, autoregulation, and cerebral venous outflow all contribute to the vascular component. Any factor that disturbs this circulation under physiological or pathological conditions may provoke an increase in ICP [6]. ICP monitoring is relevant in the treatment of many diseases, from neoplasias and traumas to infections, and its study is very important because variations in this pressure can lead to irreversible clinical pictures, such as dementia and cognitive derangements.

One of the neurological diseases that can affect ICP is epilepsy. This is characterized by spontaneous recurrent seizures caused by focal or generalized paroxysmal changes in neurological functions triggered by abnormal electrical activity in the cortex [8]. Because it involves hyperexcitable neurons, a basic assumption links the pathogenesis of epilepsy and the generation of synchronized neuronal activity with an imbalance between inhibitory (γ-aminobutyric acid [GABA]–mediated) and excitatory (glutamate-mediated) neurotransmission, in favor of the latter [7].

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Seizures and epilepsy are usually divided into two groups: partial and generalized. Partial or focal seizures have clinical or electroencephalographic (EEG) evidence of local onset and may spread to other parts of the brain during a seizure, whereas generalized seizures begin simultaneously in both cerebral hemispheres [9]. Temporal lobe epilepsy (TLE) is the most common form of partial epilepsy in adulthood [12, 20], possibly affecting at least 20 % of all patients with epilepsy [2].

The main features of TLE are:

1. The localization of seizure foci in the limbic system, particularly in the hippocampus, entorhinal cortex and amygdala [3]
2. The frequent finding of an initial precipitating injury that precedes the appearance of TLE [14]
3. A seizure-free time period following the precipitating injury known as the latent period
4. A high incidence of mesial or cornu ammonis (CA) sclerosis, i.e., a unilateral hippocampal lesion leading to atrophy, typically caused by neuronal loss and gliosis in Sommer's sector (the subiculum–CA1 transition zone) and the endfolium (dentate hilus) [15].

Most of these characteristics can be reproduced in chronic animal models of TLE, particularly the pilocarpine model of epilepsy. This model appears to be highly isomorphic with the human disease; thus, it has been used in many laboratories since its first description three decades ago [18, 19].

The systemic administration of pilocarpine, a potent muscarinic agonist, in rats promotes sequential behavioral and electrographic changes that can be divided into three distinct periods:

1. An acute period that builds up progressively into a limbic status epilepticus (SE) and that lasts 24 h
2. A silent (latent) period with progressive normalization of EEG and behavior that varies from 4 to 44 days
3. A chronic period with spontaneous recurrent limbic seizures (SRS), with increasing frequency and no remission [1, 4, 10]. The main features of the SRS observed during the long-term period resemble those of human complex partial seizures and recurs two to three times per week per animal [1, 4]. Another important feature of the pilocarpine model is the occurrence of widespread lesions, some of them localized in the same brain areas affected in TLE patients, and associated with neuronal network reorganization in hippocampal and parahippocampal regions [20].

Regarding ICP monitoring during epileptic seizures in humans, few studies were able to observe changes in this parameter in patients on continuous monitoring. A study reported an increase in ICP during epileptic seizures related to the type of seizures presented by the patient, and tonic–clonic seizures were associated with a more noticeable increase in ICP [16]. Another study showed that a generalized tonic–clonic seizure caused a sudden and massive increase in ICP in a patient with no previous medical history of seizures [17]. With regard to animal experimentation, increased ICP during sustained epileptic seizures was observed in cats [11].

In this context, ICP characteristics of individuals with epilepsy are not well elucidated, since there are few studies associating these two important neurological factors. In this work, an invasive (ICPi) and the new minimally invasive (ICPmi) [13] intracranial pressure monitoring methods were used to evaluate ICP features in rats with chronic epilepsy, induced by the experimental model of pilocarpine, which is capable of generating the main features of human TLE in these animals.

Materials and Methods

To evaluate ICP in animals with chronic epilepsy, the experimental set was divided into two groups of adult male Wistar rats: pilocarpine ($n=6$) and controls ($n=6$). For the pilocarpine group, seizures were induced by injection of pilocarpine hydrochloride (320 mg/kg, i.p.), preceded by methylscopolamine bromide (1 mg/kg, i.p.). Approximately 30 min after the injection of pilocarpine, most animals developed SE. To reduce the high mortality rate associated with tonic seizures, an injection of thionembutal (25 mg/kg, i.p.) was administered 90 min after the beginning of SE [5]. Regarding the control group, animals were treated with 0.9 % saline (0.1 mL/100 g, i.p.) and thionembutal (25 mg/kg, i.p.) to simulate the condition experienced by the pilocarpine group.

Three months after induction, when the pilocarpine group had already developed chronicity, presenting an average of 2–3 SRS per week/animal, animals from both groups were anesthetized with ketamine (95 mg/kg) and xylazine (12 mg/kg), and underwent a procedure for magnetic resonance imaging (MRI) acquisition to verify volumetric changes in the hippocampal regions. MRI sections were acquired using a 2-T Oxford Instruments® horizontal superconductor magnet, model 65310HR, which operates with a Bruker® spectrometer.

After that, they underwent surgery for the ICPmi (Braincare) and ICPi (intraparenchymatous; Codman) sensor installations on opposite sides of the parietal bone of the skull. Then, their ICPs were monitored simultaneously for 1 h, with a sampling rate of 200 Hz.

Analyses consisted of the frequency quantification of spontaneous recurrent seizures for the pilocarpine group, volume determination of the hippocampal regions using MRI techniques, short-time Fourier transform (STFT) for ICPi, and spectral frequency determinations for ICPmi and ICPi for both groups. For SRS frequency quantification, animals were monitored using video cameras 12 h per day, 5 days per week, during the light cycle (240 h monthly). This procedure began

15 days after SE onset and finished 3 months after this event. Concerning hippocampus volumetry, the perimeter of the regions of interest (ROI), i.e., the right and left hippocampus separately, were delimited in eight consecutive images, and their areas were multiplied by the slice thickness to obtain the volume. ROI selection and volume acquisition were performed using the software MRIcro. Selection of the hippocampus for analysis covered its entire rostrocaudal length between 2.30 and 6.0 mm from the bregma. Volumes for each acquisition were calculated and statistically compared between groups. All results were analyzed using one-way ANOVA followed by post-hoc Bonferroni test, with statistical significance set at $P<0.05$. STFT is a Fourier-related transform used to determine the sinusoidal frequency and phase content of local sections of a signal as it changes over time. In this case, STFT was used to define a certain behavioral pattern for ICPi frequencies in the epileptic group compared with the controls. With regard to ICPmi and ICPi spectral frequency determinations, this analysis was applied to the monitoring data to verify whether both methods were able to acquire corresponding frequency ranges.

Results

Frequency of SRS (seizures/month) in the animals treated with pilocarpine was 7.66 ± 1.46 (mean ± standard error of the mean; Fig. 1). Seizure frequency differed among the animals under study, and thus, when analyzing the group as a whole the standard deviation was 3.58 seizures/month. Nevertheless, it should be noted that the animals were monitored only during the light phase of the light-dark cycle, and possible seizures that they might present during the night were not considered in the analysis.

Concerning tissue volume measurements (mm^3) for rostral, caudal, and total hippocampus, 3 months after SE, there were statistically significant reductions in the rostral hippocampus ($P<0.05$), the caudal hippocampus ($P<0.01$), and the total hippocampus ($P<0.01$) in the pilocarpine group compared with the control (Table 1).

The spectral frequency analysis demonstrated correspondence between ICPmi and ICPi in the frequency domain for both groups (Fig. 2), indicating that the methods were capable of acquiring corresponding ranges of ICP frequencies. For STFT analysis (Fig. 3), oscillations throughout time in the ICP frequency components (fundamental frequency and harmonics) were noticeable for the epileptic compared with the control animals.

Discussion

The frequency quantification of spontaneous recurrent seizures for the animals with chronic epilepsy showed an increasing number of seizures from month 1 to month 3,

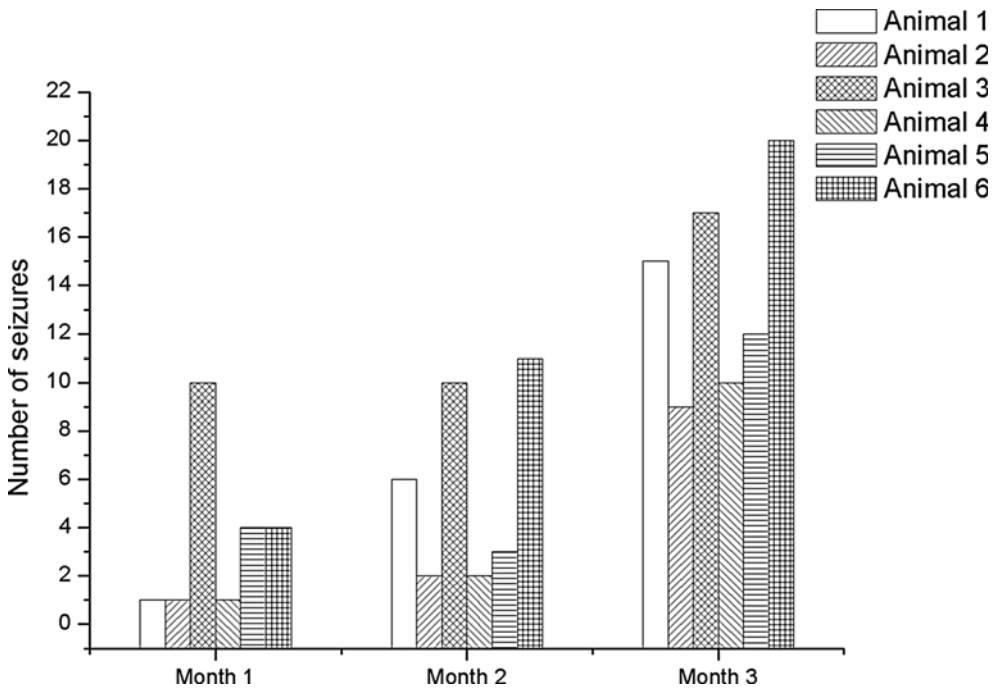

Fig. 1 Number of spontaneous recurrent seizures in each period. The whole period consists of 3 months of behavioral observations, 12 h/day (wake cycle). Observations began 15 days after status epilepticus (SE) onset

Table 1 Volume measurements (mm^3; mean ± SEM) of rostral the hippocampus (RH), the caudal hippocampus (CH), and the total hippocampus (TH) for the pilocarpine and control groups

Groups	*n*	RH	CH	TH
Pilocarpine	6	22.1 ± 1.8[a]	51.7 ± 4.6[a]	73.8 ± 6.2[a]
Control	6	26.9 ± 0.6	68.7 ± 0.7	95.5 ± 0.9

[a]Indicates statistical significance ($P<0.05$ for RH; $P<0.01$ for CH and TH) compared with the control group

when the pilocarpine group presented an average of 2–3 SRS per week/animal, a result consistent with the existing literature [4].

Results obtained using the technique of hippocampal volumetry by MRI indicated differences in the experimental group compared with the controls for all hippocampal volumes (rostral, caudal, and total); there were more marked differences in the caudal region. This hippocampal

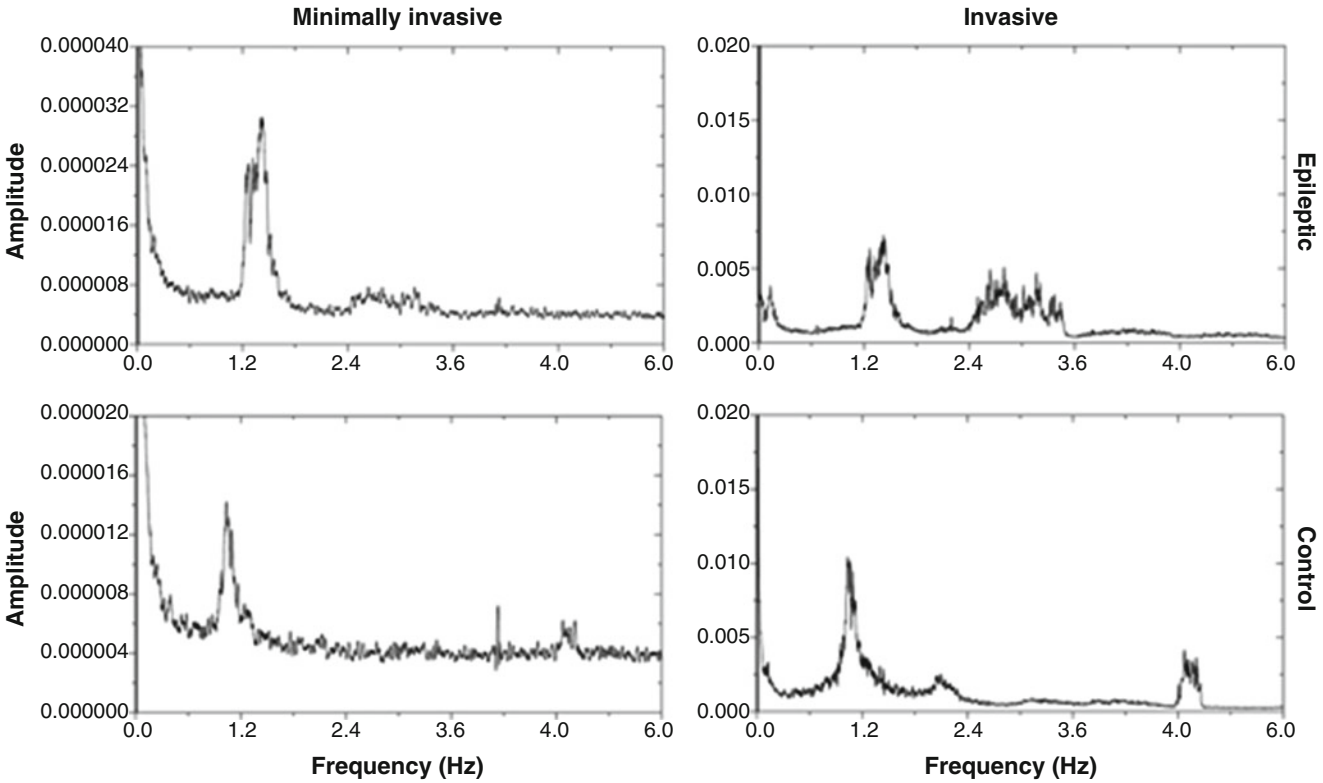

Fig. 2 Frequency spectrum analysis of minimally invasive intracranial pressure (ICPmi) and invasive intracranial pressure (ICPi) signals, demonstrating that the two methods were equally capable of acquiring corresponding ranges of ICP frequencies

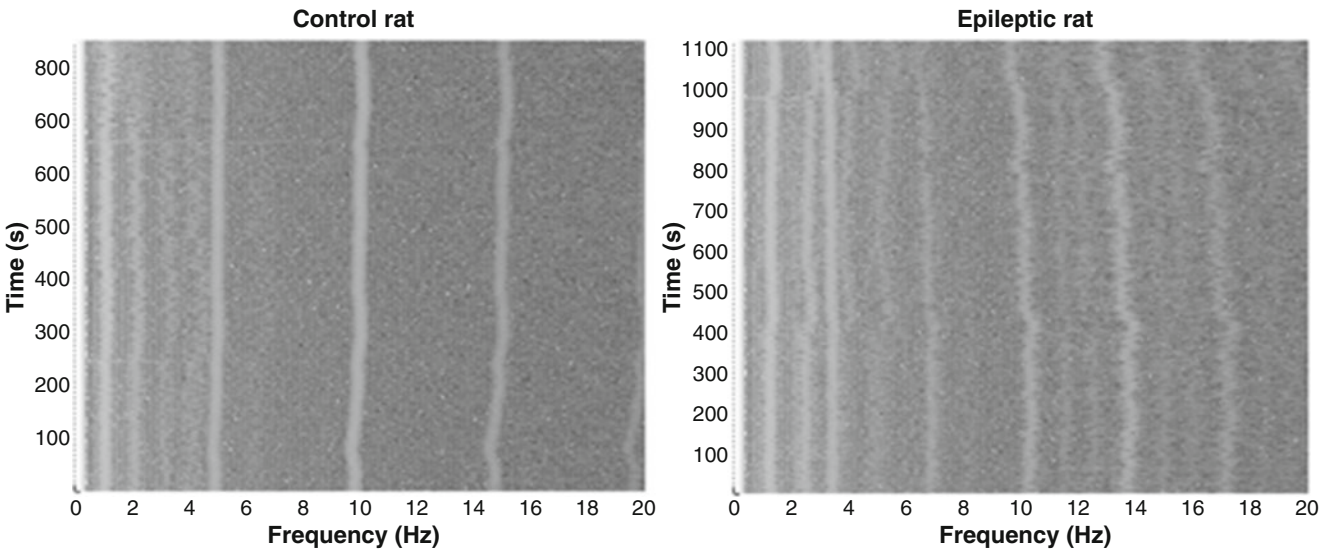

Fig. 3 Short-time Fourier transform analysis of ICPi signals. It is possible to notice oscillations and dispersions in the ICP frequency components of the epileptic animal compared with the control, which may be associated with a decrease in brain compliance and failure of autoregulation

decrease in the animals treated with pilocarpine may be related to hippocampal sclerosis in this model.

The ICP behavior of the animals with chronic epilepsy presented a characteristic of dispersion in the frequency components, which may be related to a decrease in brain compliance and failure of autoregulation. In addition, there are no reports in the literature regarding these results. Furthermore, MRI of the hippocampal region confirmed the neurological damage caused by the pilocarpine model, which may have contributed to the appearance of such ICP behavior in the animals with epilepsy.

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Conflict of Interest The authors declare that they have no conflict of interest.

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