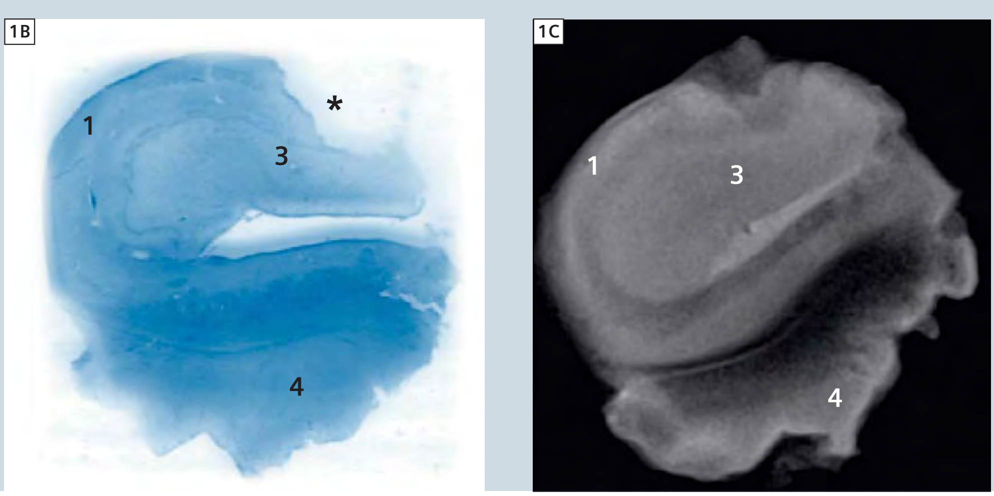
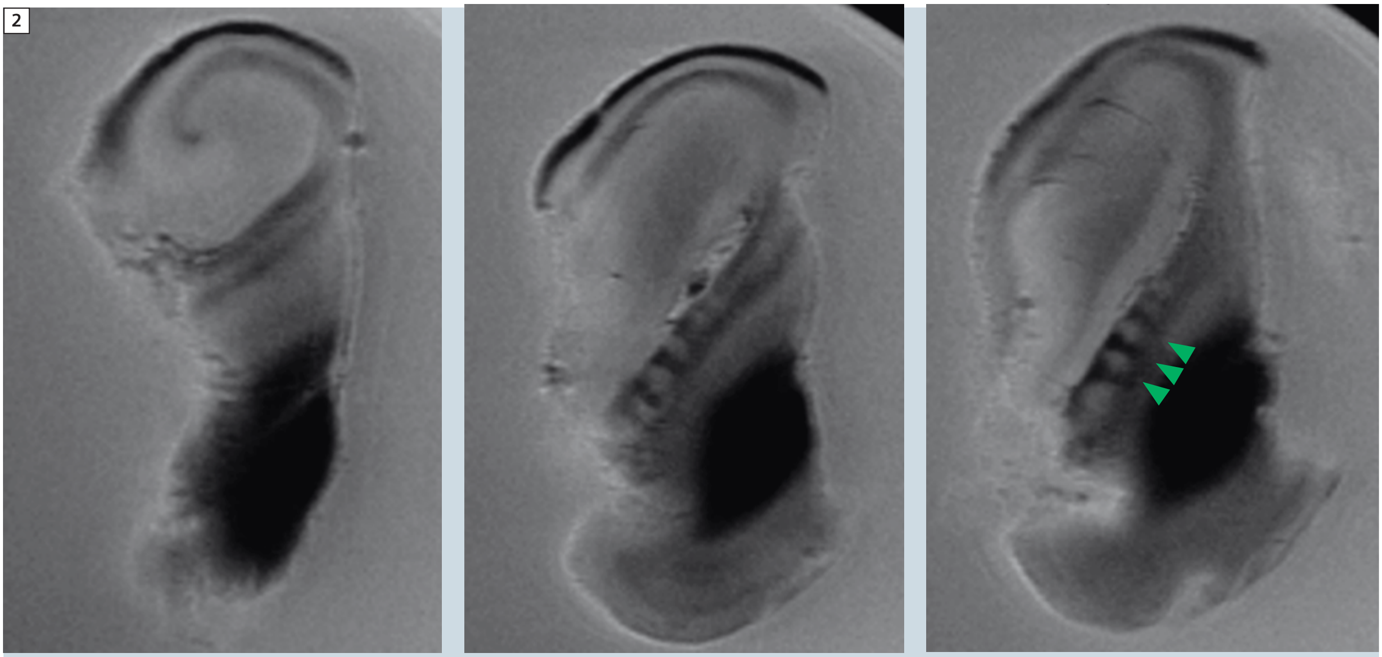
Texture Analysis and Classifiers Applied to High-Resolution MRI from Human Surgical Samples in Refractory Mesial Temporal Lobe Epilepsy

Maryana de Carvalho Alegro[1,2]; Silvia Yumi Bando[3]; Alexandre Valotta Silva[1,4]; Bruno Cunha Medeiros[1]; Felipe Brunetto Tancredi[1]; Roseli de Deus Lopes[2]; Carlos Alberto Moreira-Filho[3]; Edson Amaro Jr.[1,5]

[1]Brain Institute, IIEP Albert Einstein, São Paulo, Brazil
[2]Integrated Systems Laboratory, Escola Politécnica da Universidade de São Paulo, Brazil
[3]Department of Pediatrics; Faculdade de Medicina da Universidade de São Paulo (FMUSP), Brazil
[4]Department of Biosciences, Universidade Federal de São Paulo, Campus Baixada Santista, Brazil
[5]Department of Radiology and Oncology, FMUSP, São Paulo, Brazil



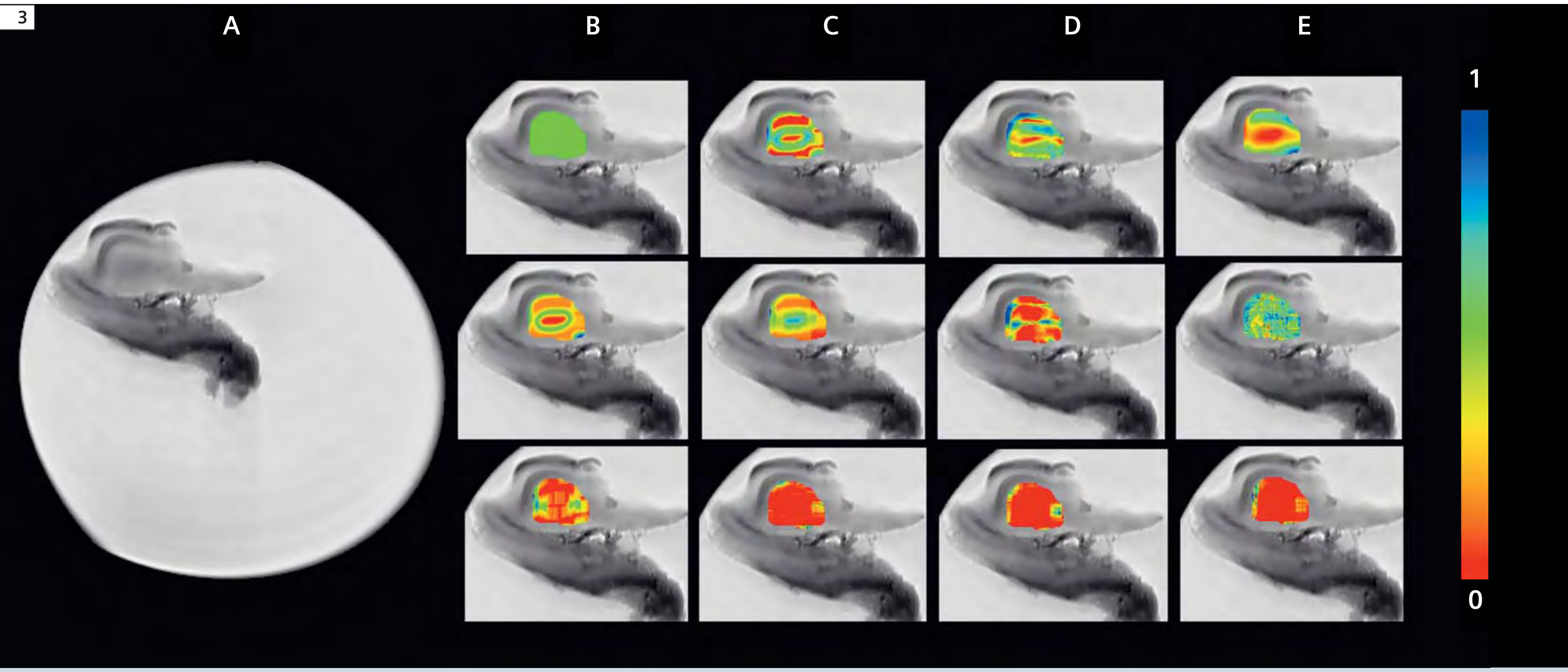
1 Hippocampi imaging scheme from surgery to pathological examination. **(1A)** Surgical view of the hippocampus. **(1B)** Pathological plate for optical examination. **(1C)** High-resolution MRI. The resected specimen is stored in a tube with formalin and high-resolution acquisitions are performed. The same material is then sliced for pathological examination. Visible hippocampal structures are marked with number in images **1A–C**: (1) CA1, (2) hippocampal head, (3) dentate gyrus, (4) parahippocampal gyrus. (*) Indicates the region where tissue was extirpated for genomic analysis.



2 Three examples of high-resolution MRI of ex-vivo hippocampi. Attained resolution allows the visualization of nervous fiber bundles crossing between different hippocampus regions (green arrowheads).

Introduction

There has been a significant increase in the number of papers using image processing techniques to improve our understanding of MR images. One family of image analysis methods is based on the relationship of a given voxel signal and the signal of its neighbors: texture analysis. Texture of an image can be characterized as the spatial distribution and spatial dependency between the pixel values of an image. Texture analysis provides a set of computational techniques that aim to mathematically characterize these distributions and dependencies. Texture features are basically numeric parameters, which are calculated by the analysis algorithms and can be fed into investigation tools, such as statistical and classification systems. They are roughly separated into four groups: statistical, structural, model-based and transform-based (see Ref. 1 for an in-depth overview of texture analysis).
Texture features do not usually carry a direct 'meaning' about the physical properties of the imaged tissue, although there are some exceptions, such as the fractal dimension [2] that approximately correlates with the 'roughness' of the image. A texture parameter alone is only a number and may be considered useless by a human observer. But sometimes it can be a powerful tool for revealing subtle changes between image samples. In fact, texture features were shown to be able to detect small signal changes in MR images, which are not detectable by human vision [3].
Although texture analysis has a long history in the medical image-processing field [4–6], there are only a few studies using MR datasets, and even fewer including patients with epilepsy, a condition characterized by high incidence in global population, varying from 1 to 3%. The most common form of human epilepsy is mesial temporal lobe epilepsy (MTLE), which affects primarily the amygdala and hippocampus, brain areas that are involved in learning and memory processes. MTLE onset is frequently associated with long febrile seizures in the early years of childhood, although some patients develop it later in life [7, 8]. The MR findings include hippocampal sclerosis: the 'shrinkage' of hippocampal formations, mainly caused by neuronal death, and associated with occurrence of disabling seizures. Some patients with MTLE may not respond to conventional non-surgical therapy, a condition known as refractory MTLE. Such patients usually undergo detailed evaluation in order to prepare for surgical extraction of the epileptogenic hippocampus. Pathological examination of resected sclerotic hippocampi reveals typical histological features in some hippocampal areas, such as gliosis and neuronal cell loss [9, 10].
Genomic analysis of the ex-vivo hippocampi samples also reveals changes in the expression of several genes, which leads to inflammatory processes [11, 12] that could also be detected immunostaining histological samples. Our group conducted comparative genomic analysis of MTLE patients with (FS) or without (NFS) febrile seizure history, which showed the correlation between hippocampal gene signature and initial precipitating injury in MTLE [13]. Ultimately, these differences could be represented in the parameters obtained by texture analysis, but not necessarily providing any hints on how the proposed



3 High-resolution MRI and some of the used texture features: a pre-processed high-resolution MRI slice can be seen in column a. Column b displays the hand drawn dentate gyrus mask in green (top), co-occurrence correlation (middle) and co-occurrence contrast (bottom). Column c displays the co-occurrence entropy (top), run-length SRE (middle) and 1st level Daubechies 4 wavelet horizontal orientation (bottom). Column d displays the fractal dimension (top), 1st order Markov random field (middle) and 1st level Coiflet 3 wavelet – diagonal orientation (bottom). Column e displays the Gabor filter (top), 5th order Markov random field (middle) and 2nd level Symlet 2 wavelet – vertical orientation. All texture features values were rescaled to the [0,1] interval.

disease mechanisms could be distinguished by texture features. On the other hand, the magnitude of the representation is large enough to induce MR signal disturbances, although too subtle to be detected by visual inspection.
Both genomic and histological techniques are widely applied in the MTLE studies, but have the drawback of being destructive procedures, preventing the samples from being used in subsequent investigations. It is yet undetermined if advanced MR acquisition and image analysis could help to better characterize each individual case.
Our goal here is to explore the application of MRI imaging for conducting 'tissue-level' studies of the sclerotic hippocampal structure. For this paper, we acquired high-resolution images of the resected specimens with a customized MRI protocol in a clinical MR system (Siemens 3T MAGNETOM Trio, a Tim system). Texture features were the main source of information for analysis, due to their aforementioned ability for detecting subtle differences in MRI images. All high-resolution images were processed in a pipeline setup to facilitate routine analysis of the surgical samples. Our first experiment was to try to discriminate between patients with febrile (FS) and non-febrile (NFS) initial precipitating injury. This differentiation by imaging is challenging since these conditions have similar clinical courses and imaging features are non specific using classical radiological assessment. In fact, such a challenge is attainable by texture analysis techniques, since only subtle morphological alterations are expected [13].

Experimental details and results

This study is part of the CInAPCe-FAPESP program, a multicenter project targeted to study epilepsy via neuroimaging (www.cinapce.org.br) performed at four main research centers equipped with 3T MR systems. Here we report findings from twelve patients with refractory MTLE who underwent surgical resection of the sclerotic hippocampus. Four patients had a clear episode of febrile seizure during childhood (2 males) and eight (5 males) had no records related to specific precipitating injury reported after careful history assessment. All surgical specimens were freshly removed, fixed in formalin solution and stored in Falcon plastic tubes.
Acquisitions were performed in a 3T MAGNETOM Trio, a Tim system (60 cm bore, 40 mT/m, 230 mT/m/s) using a surface loop coil (7 cm diameter). The Falcon tube was firmly held in the center of the coil with the aid of anti-vibration pads to avoid motion artifacts. High-resolution images were acquired using a Turbo Spin Echo (TSE) protocol, with TR 3700 ms, TE 76 ms, fat sat by IR, TF 7, FA 180°, BW 40 Hz/px, FOV 43 mm (70% AP phase oversampling), a 512 x 464 matrix, slice thickness of 1.6 mm and 32 NEX. Attained in-plane resolution was 80 µm x 80 µm and CNR (gray/white matter) 15. Figures 1 and 2 illustrate the acquisition process and hippocampus high-resolution images, respectively.
Image analysis was performed in a three-stage pipeline: preprocessing, feature extraction and analysis (see Ref. 14 for an in-depth methods description). We designed this pipeline so that future

investigations could be done using a standardized process. The preprocessing stage includes noise filtering, background segmentation and intensity normalization and is designed to reduce MRI artifact effects, such as noise and intensity non-standardization.

In the feature extraction stage texture features are calculated from the MR images. We adopted different texture features, which are computed using a texture extraction library implemented using Matlab (MathWorks, USA). This stage is computationally demanding, including the determination of statistical, transform and model-based features, resulting in a total of 158 features. Features are calculated for each pixel with the aid of a spatial sliding window. Masks were manually drawn by a neuropathologist on top of every considered MRI slice and used to guide the texture calculation process (Fig. 3). We chose the dentate gyrus (DG) area for analysis since it is one of the most affected structures in MTLE patients.

In the last (analysis) stage of the pipeline we used classification methods (Support Vector Machine – SVM, LIBSVM SVM – details in Ref. 14) to discriminate pixels belonging to either FS or NFS classes. Features are selected based on the information gain coefficient (IG), prior to classification. Only features with the highest IG are considered. We have trained/tested our SVM classifier using a leave-one-out scheme (for each iteration one subject is left out of the training phase, and SVM is performed with the remaining data). A permutation test was performed in order to validate the classification results. In the permutation test, class values are randomly permuted and reassigned to each subject; the leave-one-out routine is then performed again. This process is repeated 70 times and every time the classification accuracy reaches values equal or higher to those obtained by the original cross-validation, a counter is increased. The p-value is calculated by dividing the counter number by the number of permutations. Using this approach it was possible to correctly classify 76% of the test instances (p-value < 0.001).

Conclusions and future directions

The proposed pipeline proved to be adequate for accurate pixel classification of high-resolution ex vivo images obtained in a clinical MR system. We were able to show a significant correlation between MRI texture parameters and clinical characteristics of a group of MTLE patients. So far, and to our knowledge, it has not yet been shown that such high performance can be achieved by routine radiological reading. In fact, there is no single description of radiological signs pointing to either FS nor NFS etiology. Our findings indicate the existence of distinct characteristics between cases with (FS) and without (NFS) antecedent febrile seizure history. This is in line with a recent epidemiological study showing that MTLE cases with febrile IPI constitute, due to their clinical features, a unique phenotype, distinct from the non-febrile cases [15]. We are now investigating whether the same texture analysis and SVM approach holds the same performance when analysing data from pre-surgical MRI images.

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Contact
Edson Amaro Jr, M.D., Ph.D.
Brain Institute
IIEP Albert Einstein
Av Albert Einstein 627/701
05652-900, São Paulo, SP
Brazil
Phone: +55-11-2151-3727
Cell phone: +55-11-8122-7201
Fax: +55-11-2151-0273
edson.amaro@gmail.com

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