Animal model

The epilepsy induced in the rat by lithium pilocarpine (Li-Pilo) constitutes an animal model of human mesial temporal lobe epilepsy. Neuronal damage is mainly detected in hippocampus, thalamus, piriform cortex, entorhinal cortex, and neocortex. At present, magnetic resonance imaging (MRI) is the most sensitive imaging method for the study of mesial temporal lobe epilepsy, but the examination is often restricted to the detection of hyperintensities. In previous studies, we used MRI to explore the morphological changes resulting from an injection of Li-Pilo that leads to epilepsy. In order to improve the predictive value of MRI images, we performed a texture analysis of MRI images combined with a discriminant analysis. The results presented here indicate that this procedure can detect defects that cannot be visualized by classic examination and permits a more correct classification of the images.

Materials and methods

MRI protocol

MRI images were recording using an MRI scanner operating at 4.7 tesla (SMIS, UK). The rats were anaesthetized for MRI by an intramuscular injection of 37 mg/kg keta-
mine and 5.5 mg/kg xylazine. A T2-weighted spin-echo fast-imaging method sequence (repetition time [TR]=3800 ms and echo time [TE]=80 ms) was used with 4-cm field of view, a 256×256 pixel matrix, 1-mm thickness, upon coronal slices of the whole brain.

Animals and Li-Pilo protocol

Eleven 21-day-old, male, Sprague-Dawley rats were used for the experiments. The images of the 11 rats obtained before the injection of Li-Pilo served as control. All the rats first received lithium chloride (3 mEq/kg) intraperitoneally. After 18 h, the rats received a subcutaneous injection of pilocarpine (30 mg/kg) and 30 min later 1 mg/kg methylscopolamine intraperitoneally, in order to reduce the peripheral consequences of pilocarpine administration. Two hours after onset of status epilepticus (SE), the rats received 2 mg/kg diazepam by deep intramuscular injection in order to improve their survival. Images of all the rats were performed 24 h after onset of SE.

Texture analysis

Conventional texture analysis was performed using statistical methods, mostly based on first-order and second-order histograms derived from the co-occurrence matrix, which describes the spatial gray level dependencies. Another possibility is the run-length matrix, which is the matrix of the run-length frequency occurring in the image for a certain angle of sight (lines of the same pixel level). This method has been fully described by Haralick. The co-occurrence matrix is based on the probability that pairs of pixels with a given level will appear. For each orientation (0°, 45°, 90°, and 135°) and for each distance between two pixels forming a pair, a number of co-occurrence matrix parameters may be calculated: contrast (an uneven texture provides large/high contrast values); correlation (relationship between two pixels); homogeneity (uniformity of the gray levels); and entropy (coarse-grained quality of the texture). The software MaZda was used to analyze the texture of the digitized images within all regions of interest (ROI) and yielded 300 parameters.

Statistical analysis

The statistical analysis was carried out using software from Statistica, Statsoft Inc. Discriminant analysis was used for multigroup classification. Using stepwise analysis, we checked the ability of each texture parameter to discriminate between two groups of ROIs, ie, presence or absence of lesions in piriform or entorhinal cortices. As a preliminary step, we determined the most important parameters that best discriminated the “lesion” ROIs from the “safe” ROIs observed before the Li-Pilo protocol. The question to be answered here is whether the two groups are well distinguished on the basis of the set of texture parameters. If the discrimination is successful on the basis of the set of selected parameters, it makes sense to classify particular piriform or entorhinal cortices in terms of group membership, ie, in terms of into which group they are most likely to be classified. The search for hidden defects could then be undertaken in the nonmodified images, obtained after the Li-Pilo protocol, in order to discriminate between lesion and safe ROIs.

Results

In all 21-day-old rats (n=20), pilocarpine injections led to SE within about 50 min. However, only 80% of rats were still epileptic after a mean delay of 70.2±24.6 days (mean±SD). MRI images obtained before Li-Pilo treatment were considered as control group images (Figure 1) (64 ROIs were used for the texture analysis). Among the 20 rats followed for 4 months, 16 exhibited seizures, whereas 4 did not. Retrospectively, three groups of rats could be characterized according to type of images and the possibility of late epilepsy:

- **Group A**: 6 rats with obvious lesions characterized by a hypersignal on T2-weighted images in the piriform or entorhinal cortices 24 h after the SE (Figure 1; 44 ROIs were used for texture analysis); all these rats exhibited late epileptic seizures.
- **Group B**: 4 rats with control-like images (without any hypersignals), as shown in Figure 1, which did not present late epilepsy (34 ROIs were used for texture analysis).
- **Group C**: 10 rats with control-like images (without any hypersignals), as shown Figure 1, but which subse-
quently became spontaneously epileptic (80 ROIs were used for texture analysis). Therefore, the conventional MRI study could not predict the fate of the 10 rats in group C, which did not display visible lesions in their brain images 24 h after SE, but subsequently became epileptic.

The results of the texture analysis yielded 200 texture parameters in each ROI. Preliminary discriminant analysis yielded a classification function corresponding to the control group or group A. Each function was a linear combination of the features (or texture parameters) that yielded the best discrimination. For a given ROI, described by the texture parameters, a classification score was calculated from the classification functions. Each ROI was then classified into one group or the other, according to the highest classification score. The above classification process was then used as a basis for prediction for the 114 apparently normal ROIs from the 57 brain slices of the rats in groups B and C. The resulting classification gave 84 control ROIs and 30 lesion ROIs. Indeed, only 2 rats had control ROIs and were safe (group B). About 50% of the lesion ROIs of the other 12 rats were distributed bilaterally (10 rats in group C and 2 rats in group B). During the 4 months’ clinical follow-up, 10 rats became epileptic and 4 rats remained nonepileptic, among which 2 had been incorrectly classified as epileptic (Table I).

**Discussion and conclusion**

The MRI study that was based only on the presence of hyperintensity signals in the piriform and entorhinal cortices predicted 6 late chronic epilepsy and 5 safe rats. This missed latent disease in 3 rats. The combined texture and discriminant analyses that were based on pixel pattern abnormalities selected 3 texture parameters that characterized structural abnormalities relevant to the hypersignal, both in the modified images of 6 rats and in the images of 4 rats with apparently nonmodified images, predicting the late chronic epilepsy in 10 rats. The classification based on early texture abnormalities in the piriform and entorhinal cortices improved the results of the regular MRI study.

**Human applications in AD**

The method of gray-level dependence histograms (GLDH) as defined by Chetverikov for 2D and generalized to 3D by Kovalev and Petrou leads to derived features of texture anisotropy from MRI data. The aim...
was to evaluate Alzheimer’s disease (AD) patients for a correlation between the anisotropic features and their score on the Mini-Mental State Examination (MMSE), which is routinely used to help diagnose AD.\textsuperscript{11}

**Methods**

Two groups of subjects were investigated and analyzed in this study: 12 control volunteers and 13 AD patients. The control group was matched with the AD group in terms of age and gender. The mean age (range) at time of investigation was 56.77 (39-72) years for the AD patients and 58.33 (47-72) years for the control volunteers.

MRI T\textsubscript{1}-weighted images with coronal orientation were recorded for each subject. Each data set had 180×180×124 pixels and the voxel size was 0.9375×0.9375×1.5 mm. The scans were segmented to isolate the brain from external structures (eyes, ventricles, bones, etc).\textsuperscript{12} The brains were further segmented to isolate the white and gray matter, as well as the border between the two types of tissues. Because the texture analysis technique effectively counts the number of pairs of voxels that appear in the same relative position and have certain fixed gray values, the relative gray values of the voxels are extremely important. Thus, a normalization set is used in order to have the same relative gray level values for different scans: the smallest gray-level value is assigned to 1 and the highest to 255 for the segmented scan; 0 is assigned to the voxels that do not belong to the ROI.

**3D texture representation: isotropy or anisotropy**

A coordinate system is defined as a cube of data cube in which the \(x\) and \(y\) axes form the plane of each slice, and the \(z\) axis is perpendicular to each slice. The azimuthal angle \(\phi\) is measured on the \(x,y\) plane away from the direction of the \(x\) axis. The pair of values \(\phi, z\) defines a unique orientation in 3D space. We can then calculate the quantity \(h(\phi, z; d)\). One component of \(h\) is the number of pairs of voxels that are at distance \(d\) from each other, along the direction \(\phi, z\) with one member of the pair having a gray value \(k\) and the other \(l\). If the data are isotropic, the function \(h\) must be independent of direction and therefore a 3D representation is a sphere. Any deviation from this shape indicates anisotropy in the data. The 3D representation for a fixed distance is a closed digital surface, which is called an indicatrix. Projections of the orientation histograms can be obtained as illustrated in Figure 2 for a control subject and an AD patient.

**Feature extraction**

Three features are used to analyze the shape of the 3D indicatrix:\textsuperscript{9} the anisotropy coefficient, the integral anisotropy measure or standard deviation, and the local mean curvature. Another set of features can be extracted by expanding the indicatrix in terms of spherical harmonics. The coefficients of such an expansion can characterize any 3D closed surface: coefficient \(A_{0,0}\) is the mean radius of the indicatrix; any other nonzero coefficient represents different types of anisotropy. Anisotropic features were extracted from four brain regions: the whole brain, white matter, gray matter, and the border between gray and white matter. In every single region, five different distances \(d\) were used: 0.9375, 1.5, 2, 2.5, and 3 mm.

**MMSE score and correlation with the isotropy coefficient**

The MMSE score is used to detect dementia. The maximum score is 30 (typically above 29 for healthy volunteers). Scores between 10 and 24 are considered to indicate mild-to-moderate dementia cases, and scores below 10 indicate severe dementia. The scores obtained in the AD patients (named AD\textsubscript{1} to AD\textsubscript{13}) and the control volunteers (named CO\textsubscript{1} to CO\textsubscript{12}) are displayed in Table II. Two of the scores do not match the clinical diagnosis: AD\textsubscript{3} and CO\textsubscript{2}.

While many features correlate well with the MMSE scores, Figure 3 illustrates the best correlation (-0.876) with the MMSE score, which was obtained for the feature \(A_{1,1}\) in gray matter for a distance of 0.9375 mm. Subject AD\textsubscript{3} is interesting because this patient was imaged before the onset of the first clinical symptoms, at a time when there may have been ongoing structural brain changes.
Discussion and conclusion

The GLDH method can be used to produce many features that strongly correlated with the MMSE scores when applied to the gray matter components of the MRI T1 scans. The features computed reflected the microtextural properties of the brain, i.e., the texture anisotropy at scales of the order of 1 mm, rather than the shape of each ROI. Moreover, they correlate better with the condition of the subject rather than with age. In general, the AD brains presented greater anisotropy in their gray matter texture than the control brains. Both 2D features and 3D features correlate with the MMSE score, indicating that the information is already available in each individual layer.13

Human applications in schizophrenia

Texture analysis can also provide feature parameters for different classes, which can then be used for classification. Generalized 4D co-occurrence matrices have been used to analyze the 3D MRI T2-weighted brain images from controls and patients with schizophrenia. The ROI approach has a number of potential problems: inter- and intraoperator reproducibility; difficulties detecting neuroanatomic boundaries; and the requirements that are of interest have to be specified from the outset. This approach does not need prior hypotheses and, because it is automated, reproducibility and comparability are ensured.

### Table II

| Alzheimer’s disease | Controls |
|---------------------|----------|
| Patient | MMSE score | Subject | MMSE score |
| AD1     | 25        | CO1     | 30          |
| AD2     | 8         | CO2     | 28          |
| AD3     | 30        | CO3     | 30          |
| AD4     | 25        | CO4     | 29          |
| AD5     | 23        | CO5     | 30          |
| AD6     | 25        | CO6     | 30          |
| AD7     | 28        | CO7     | 29          |
| AD8     | 22        | CO8     | 30          |
| AD9     | 19        | CO9     | 30          |
| AD10    | -         | CO10    | 30          |
| AD11    | 14        | CO11    | 30          |
| AD12    | 24        | CO12    | 30          |
| AD13    | 12        |         |             |

Methods

Two groups of subjects were investigated and analyzed in this study: 19 control subjects and 21 patients with schizophrenia. The controls were matched for age, gender, and social class. 3D MRI T2-weighted images were collected for each subject. Each data set consisted of slices with a 0.856-mm spatial resolution with interslice distance of 3 mm. The images were segmented such that only the brain component was extracted for further analysis. The anisotropic sampling of the data along the z axis (interslice direction) is handled by an appropriate scaling factor of 3.5 (3/0.856) for all data sets. For the texture analysis, we use generalized co-occurrence matrices.14

3D texture analysis

The co-occurrence matrix is a generalized histogram, which records the frequency with which a certain combination of characteristics appear in the relevant position. Usually the main characteristic used is the gray value of the image, but other features can be used, such as gradient magnitude or relative orientation of the gradient vectors. Because they are independent of rotation and translation of the data, co-occurrence matrices offer...
descriptors that include these properties. The calculated co-occurrence matrix was \( w[g(i), g(j), a(i,j), d(i,j)] \), where \( w \) is the frequency of the occurrence of a voxel pair \( i,j \), with gradient magnitude \( g(i) \) and \( g(j) \) respectively, an angle \( a(i,j) \) between their gradient vectors, and a distance \( d(i,j) \) from each other.

**Results**

Three series of experiments were conducted: one considering the whole brain, one considering the bottom half of the brain, and one considering the bottom quarter of the brain. The division of the brain was performed by identifying the slices that are anatomically most similar to slices 12 and 24 of the anatomical atlas of Talairach and Tournoux. In each experiment, each element of the co-occurrence matrix was tested as a class discriminator according to the \( t \) test. The feature were selected by thresholding the \( t \) values using various limits. The classification was then performed using Statistica software.

For the whole brain, the best results were obtained by retaining the features with \( t>4.5 \), and 14 subjects was misclassified. Using the bottom half of the brain, there were too many features with \( t>4.5 \) and so only the features with \( t>5.5 \) were used, and only 7 subjects were misclassified. When the bottom quarter of the brain was used, the features were so good that only features with \( t>7.5 \) were retained and only 2 subjects were misclassified, as illustrated in Figure 4.

**Conclusions**

The most significant conclusion is that the brains of patients with schizophrenia show structural differences from the brains of the control subject. Moreover, from the three series of analysis performed, it appears that these differences are located in the bottom quarter of the brain. Finally, it was demonstrated that the co-occurrence matrices could characterize the two classes of subjects with 90% accuracy using 3D T2-weighted MRI.

**Perspectives**

Texture analysis is a new approach for image analysis. Once the pharmacological aspect in the rat model is clearly demonstrated, extension to potential applications for humans can be considered. In fact, brain plasticity could be assessed with such a technique in brain diseases such as epilepsy, dementia, and schizophrenia. Drug effects could also be investigated in order to evaluate whether brain anisotropy or asymmetry varies during drug therapy. Finally, such an analysis could be correlated with neurocognitive tests to measure improvements in subjects’ performance.

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**Análisis de la textura del cerebro: desde los modelos animales a las aplicaciones en el hombre**

Las imágenes de resonancia magnética (IRM) se utilizan ampliamente para estudiar cerebros in vivo, tanto a nivel experimental en modelos animales como para orientaciones diagnósticas en el hombre. Gran parte del valor de las IRM se relaciona con el hecho que el contraste del tejido blando se acentúa por la variación importante de los tiempos de relajación $T_1$ y $T_2$ entre los tejidos. Puede ser posible el empleo de una aproximación alternativa que no se basa en la medición absoluta de los tiempos de relajación. En general, las texturas son patrones visuales complejos compuestos de entidades o subconfiguraciones que tienen brillo, color, ángulo, tamaño, etc., características. De esta forma, una textura puede considerarse una agrupación de unidades semejantes en una imagen. Las propiedades de la subconfiguración local determinan la percepción de luminosidad, uniformidad, densidad, aspereza, regularidad, alineación, frecuencia, fase, orientación, grosor, aspecto aleatorio, finoza, tersura y granulaciones. El presente artículo se propone ilustrar cómo se puede aplicar el análisis de la textura en modelos animales y en clínica humana, al igual que en la investigación de futuras aplicaciones farmacológicas en el hombre. Se presentan tres estudios diferentes de IRM: (1) en animales, utilizando el modelo de rata epiléptica por pilocarpina, (2) en pacientes con enfermedad de Alzheimer y (3) en pacientes con esquizofrenia.

**Analyse de la texture du cerveau : des modèles animaux aux applications humaines**

L'imagerie par résonance magnétique (IRM) est largement utilisée dans les études du cerveau in vivo, tant sur le plan expérimental dans les modèles animaux que dans une visée diagnostique chez l’homme. Une grande partie de la valeur de l’IRM est liée au fait que le contraste du tissu mou est accentué par la variation importante des temps de relaxation $T_1$ et $T_2$ dans les divers tissus. Il est peut-être possible d’utiliser une autre approche, qui ne s’appuie pas sur la mesure absolue des temps de relaxation. De façon générale, les textures résultent de motifs visuels complexes composés d’entités et de sous-motifs ayant une brillance, une couleur, un angle, une taille, etc., caractéristiques. Une texture donnée peut donc être considérée comme un groupement de similitudes au sein d’une image. Les propriétés d’un sous-motif local sont à l’origine de ce qui est perçu en termes de luminosité, d’uniformité, de densité, d’inégalité, de régularité, de linéarité, de fréquence, de phase, de direction, de grossièreté, d’aspect aléatoire, de finesse, d’aspect lisse et de granulation. Le présent article se propose d’illustrer comment l’analyse de la texture peut être utilisée dans des modèles animaux et dans des applications cliniques humaines, ainsi que dans la recherche d’applications pharmacologiques futures chez l’homme. Trois études IRM y sont présentées, l’une menée dans un modèle animal de rat rendu épileptique par la pilocarpine, la seconde chez des patients atteints de maladie d’Alzheimer et la troisième chez des schizophrènes.