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Unsupervised Segmentation of Brain Regions With Similar Microstructural Properties: Application to Alcoholism

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Abstract— **In this work, a novel brain MRI segmentation approach evaluates microstructural differences between groups. Going further from the traditional segmentation of brain tissues (white matter –WM-, gray matter –GM- and cerebrospinal fluid –CSF- or a mixture of them), a new way to classify brain areas is proposed using their microstructural MR properties. Eight rats were studied using the proposed methodology identifying regions which present microstructural differences as a consequence on one month of hard alcohol consumption. Differences in relaxation times of the tissues have been found in different brain regions ($p<0.05$). Furthermore, these changes allowed the automatic classification of the animals based on their drinking history (hit rate of 93.75 % of the cases).**

I. Introduction

Alcoholism is an important psychiatric disorder among European population (currently is top 3 of death causes) [1]. Co-morbidity in alcoholism is common with disorders such as depressions, anxiety, bipolar disorders, psychosis, and antisocial behavior [2]. Heavy alcohol intake has been associated with both structural and functional changes in the central nervous system [3].

Neuroimaging is being increasingly used in the diagnostic and longitudinal study of mental disorders, becoming an important tool in clinical environments. Anatomical, functional, and biochemical changes in the brain of alcoholic patients have been observed: a reduction of cortical thickness [4], a reduction of white [5, 6] and gray matter [6, 7], an increase in cerebrospinal fluid volume [5], and a gray matter dysfunction [6, 7].

It is important to note that all findings mentioned above are based on the study of structural changes in the brain. For this purpose, a robust MRI brain tissue segmentation is needed. In fact, segmentation of brain images has been an important challenge in the last few years and numerous approaches have been proposed [8-10], being divided in supervised and unsupervised classification techniques.

Usually, unsupervised segmentation algorithms statistically model the distribution of MRI intensities, assuming in most of the studies three different brain tissues (white matter, gray matter, and cerebrospinal fluid) and studying their differences from a structural point of view. However, multimodal imaging methods allow more precise measurements of brain microstructure. Physical properties of water that govern MRI contrast can be used to characterize tissue attributes. Different quantitative parameters are related to the contrast between tissues. Relaxation times (T1 and T2), magnetization transfer saturation (MT) and parameters derived from Diffusion Tensor Imaging (DTI) such as fractional anisotropy (FA, an index of fiber coherence) or mean diffusivity (MD) are widely used.

This work has two main objectives. Firstly, a novel segmentation method is proposed that complements traditional brain segmentation methods with a novel tissue classification based on similar microstructural properties derived from quantitative multimodal MRI. Secondly, the effects on brain microstructure associated to hard alcohol intake in a genetic rat model of alcoholism have been studied using the developed methodology.

II. Methods

A. Animals

Drug naïve male rats of the Sardinian-marchigian alcohol preferring msP line [11] were obtained from the breeding facility at the University of Camerino, Italy. The breeding has continued for over 35 generations. Alcohol consumption in msP rats is > 5 g/kg/day.

In total, 8 subjects were considered for this study. Animals were individually isolated each other for 30 days with 2 bottles: one full of water and the other full of EtOH diluted to 10% in water. Dissolution and water consumptions and weight were registered.

MR scans were acquired before and after alcohol consumption in order to evaluate changes between conditions.

B. Data acquisition

All images were acquired using a Bruker Biospec 7T (Bruker Biospin, Ettlingen, Germany). Anesthetized animals were placed in a modified saddle coil integrated within a customized stereotaxic animal holder. This allows precise positioning of the animal with respect to the coil and the magnet and avoids movement artifacts.

Fifteen axial slices were planned for every subject (FOV=32 × 32 mm^2, matrix size = 128 × 128, in-plane resolution = 0.125 × 0.125 mm^2, slice thickness = 1 mm).

Diffusion Tensor Imaging data was acquired using an Echo Planar Imaging diffusion sequence, with 30 uniform distributed gradient directions, b = 670 s/mm^2, with two non-

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diffusion weighted images, repetition time (TR) = 4000 ms, echo time (TE) = 23 ms.

T2 map images were acquired using a multi-slice multi-echo sequence (TR = 6000 ms, TE = [12 24 36 48 60 72 84 96 108 120 132 144 156 168 180 192 204 216 228 240 252 264 276 288 300 312 324 336 348 360] ms).

T1 map images were acquired using a Rapid Acquisition with Relaxation Enhancement (RARE) sequence with variable repetition time (TE = 12.61 ms, TR = [155 250 400 800 1600 3500 6000] ms).

In order to facilitate spatial preprocessing steps, a three-dimensional (3D) image was acquired using a RARE sequence (field of view = 32 × 32 × 16 mm^3, matrix size = 256 × 128 × 64, voxel size of 0.125 × 0.25 × 0.25 mm^3, TR = 1500 ms, TE = 9 ms).

C. Preprocessing

All same subject's modalities were realigned using SPM 8 (Wellcome Trust Centre for Neuroimaging, University College London, London, UK). Rigid transformation parameters were calculated using non-diffusion volume (DTI), the largest repetition time volume (T2 map) and the shortest echo time volume (T1 map).

Three-dimensional images were re-scaled by a factor of 10 and skull stripped using BET (FMRIB Centre, University of Oxford, Oxford, UK) [12]. Before brain extraction, images were rescaled in the anterior–posterior direction by a factor of 0.5, which made the rat brains more spherical. The output image was reversed to original size and shape. Calculated mask was used to delete non-brain tissue from other modalities.

Spin-lattice relaxation time maps (T1 maps) and spin-spin relaxation time maps (T2 maps) were calculated using an in-house script written in MATLAB 7.1 (The MathWorks, Inc., Natick, MA, USA) fitting the data using a non-linear curve fit algorithm.

DTI were corrected for motion and eddy current distortion and fitted of local diffusion tensor using an available tool in FSL library for this purpose. From the diffusion tensor components, Fractional Anisotropy (FA) was determined.

In order to reduce confounding factors coming from the complex and heterogeneous anatomical structure of the brain couple with the inter-individual variability and the relatively low spatial resolution attainable (partial volume effects), a coronal slice located in a comparable brain position in each subject is only used in the study.

D. Gaussian Mixture Model Definition

There is a long tradition in the statistical literature of using finite mixture models (FMM) to perform probabilistic clustering allowing overlap of the clusters and handling uncertainty about cluster membership in a probabilistic way.

FMMs are weighted sums of a finite number of parametric probability density functions (pdfs) called component densities. A component models the probability of the data to belong to a certain class in an unsupervised classification problem. As MRI intensities distributions can be modeled as a Gaussian distribution, Finite Gaussian Mixture (FGM) models are widely used in MRI brain segmentation.

FGM represents the probability of an intensity value (x) given a set of parameters (Φ) and is defined as (1):

$$p(x|\Phi) = \sum_{i=1}^{k} \alpha_i g\left(x\middle|\mu_i,\Sigma_i\right) \quad (1)$$

where k is the number of the components in the model and α_i are mixing parameters that models the prior probability of that class ($\sum_{i=1}^{K} \alpha_i = 1$) while $g\left(x\middle|\mu_i,\Sigma_i\right)$ is a Gaussian probability function defined by its mean μ_i and covariance matrix Σ_i that represents probability of belonging to a specific class.

The three microstructural parameters previously calculated have been used in the microstructural study of the brain. Let $x = \{T1(\vec{r}), T2(\vec{r}), FA(\vec{r})\}$ be a 3-dimensional vector which represents the three microstructural values (T1, T2, FA) in a position of the brain $\vec{r}$. $X = \{x_1, \dots, x_m\}$ is a random variable that represents m positions in the brain of a subject. D denotes all the data observed in N subjects ($D = X_1 \cup X_2 \cup \dots \cup X_N$). The set of parameters has been estimated using D.

Using Expectation Maximization (EM) algorithm [13,14], maximum-likelihood parameters are obtained by the maximization mixture log-likelihood. The EM algorithm converges in parameter space to a local maximum of the objective function, but is no guarantee of convergence to global maximum. For avoiding convergence to local maximum, algorithm is repeated 100 times with different initial parameters. The set of parameters with the largest likelihood is assumed.

Since the number of components in the model is unknown it has to be estimated. Penalized likelihood methods have often been used in model selection in mixture problem. In our case, we decided to use Bayesian Information Criterion (BIC)[15]. BIC was evaluated in models from 1 to 30 components. Optimal value (k^*) is chosen using the minimum value of k to which BIC has suffered a 99% reduction of the difference between its maximum value and its asymptotic value. Using this criterion, it is assumed that model does not get complicated for improving convergence in 1%. Also diagonal and unconstrained covariance matrices have been studied for modeling the data.

Finally, each voxel is classified in one of the components depending on the largest posterior probability of belonging to every component.

In Figure 1, the proposed tissue classification framework is showed.

E. Subject Classifier

Having defined the Finite Gaussian Model, a classifier based on linear discriminant analysis (LDA) is implemented and validated using a leave-one-out strategy. The classifier tries to distinguish between the two time points or conditions (before and after alcohol intake).

All subjects were characterized before and after alcohol intake by its mean microstructural parameter in each cluster. Both situations were compared by performing a t-test using as paired samples every microstructural mean value of each

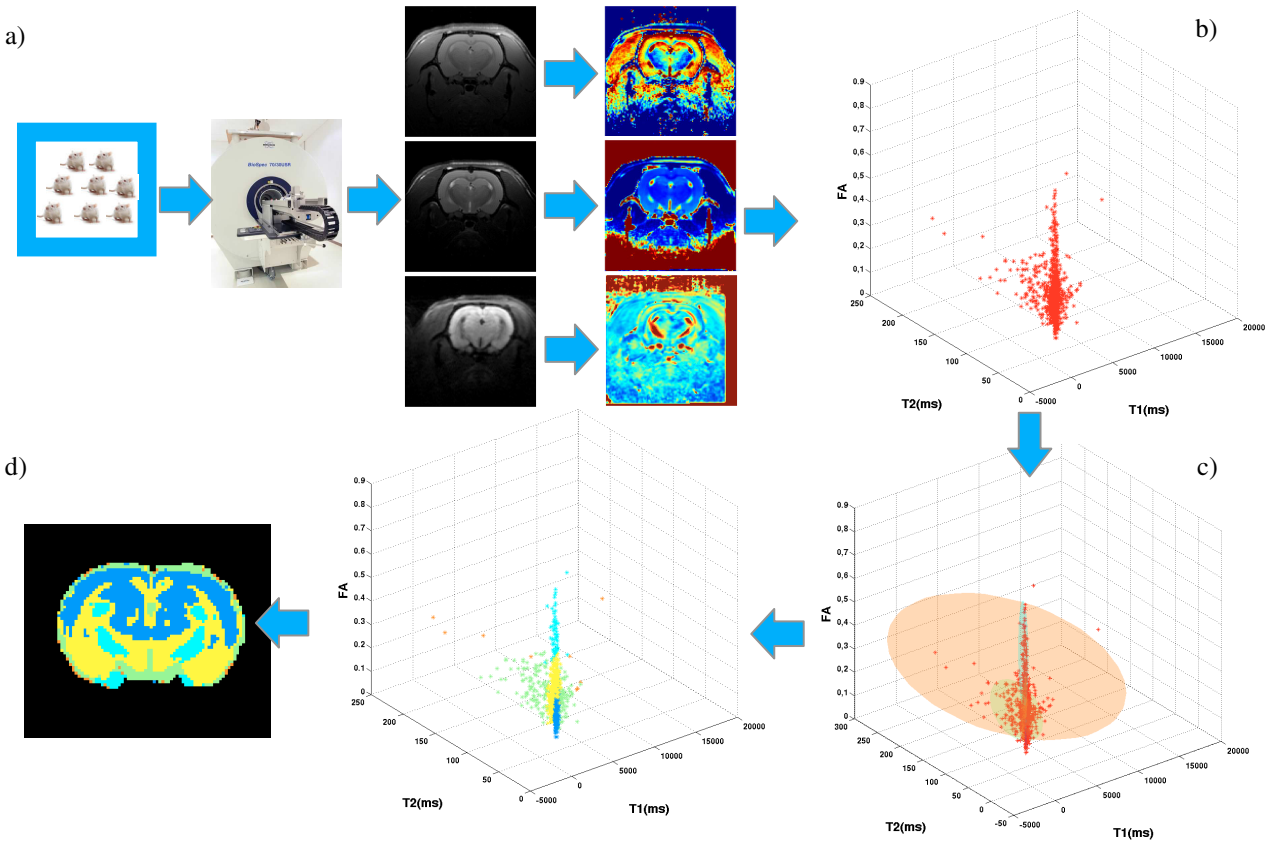

Figure 1. Framework. a) MR acquisition (T1 map, T2 map and DTI). b) Realignment and map calculation. Definition of subspace *D*. c) Model estimation with 5 components using EM algorithm. Each ellipsoid is related to every component of the model. d) Each observation (voxel) is labeled with its maximum posterior probability of belonging to each component.

cluster before and after alcohol intake. Parameters which showed a statistically significant difference ($p < 0.05$) were used as an input for the classifier.

Goodness in the classification of the data is validated using a leave-one-out strategy. Each one of sixteen data sets (eight subjects and two conditions) is classified using the rest of them as training sample. Once all sets have been classified in one of the conditions, the hit rate or accuracy is defined as the ratio between the number of correctly classified sets and the total number of sets.

III. RESULTS AND DISCUSSION

In Figure 2, BIC values for diagonal and unconstrained covariance matrices are represented for different *k* number of clusters. It is noteworthy to mention that BIC for unconstrained matrices is slightly lower than for the diagonal case. This is in concordance with the results obtained by Desco et al. [16] who suggest that unconstrained covariance matrices allow better modeling of voxels containing a partial volume effect.

Using the proposed criterion to define the optimal number of clusters, in k=14 a reduction of 99% of the difference between the maximum and the asymptotic value is observed.

Once this point has been clarified, FGM model is defined using EM algorithm by 14 components and unconstrained covariance matrices.

Posterior probability of belonging to every component of each voxel is calculated. Each voxel is labeled depending on the largest posterior probability. In Figure 3, a map of the labels in a subject before and after alcohol intake is showed.

Each cluster can be characterized using the three mean microstructural values (T1, T2, FA). Standard deviation of values in a voxel is highly related with the width of the Gaussian of the component.

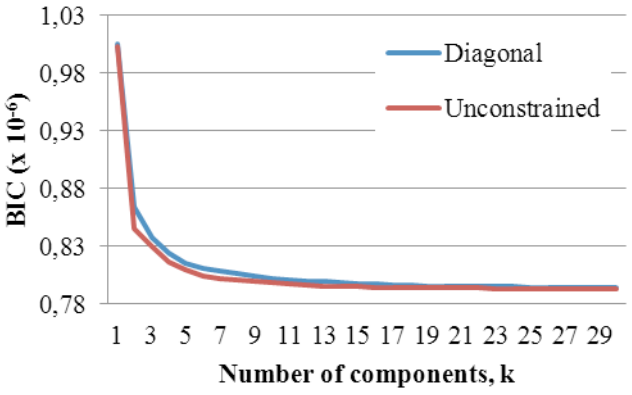

Figure 2. Bayesian Information Criterion (BIC) as a function of the number of clusters. In red, using a unconstrained covariance matrix. In blue, using a diagonal matrix.

Afterwards, a LDA classifier is implemented for clusters that present a statistically significant difference between the two time points (before/after alcohol intake) in their microstructural composition. Using a common p threshold ($p<0.05$), T1 maps present differences in clusters 2 ($p=0.047$), 3 ($p=0.039$), 4 ($p=0.024$) and 5 ($p=0.024$), while T2 maps show statistically significant differences in clusters 9 ($p=0.006$) and 12 ($p=0.002$). Fractional anisotropy did not present any difference using this presented model.

Using the model with 14 components, the hit rate is 93.75% of the cases, i.e. new subjects would be well-classified according to the evaluated microstructural parameters. High success rates are observed partially due to the inputs of the LDA algorithm, as they are microstructural values which have been chosen statistically different before and after hard alcohol consumption.

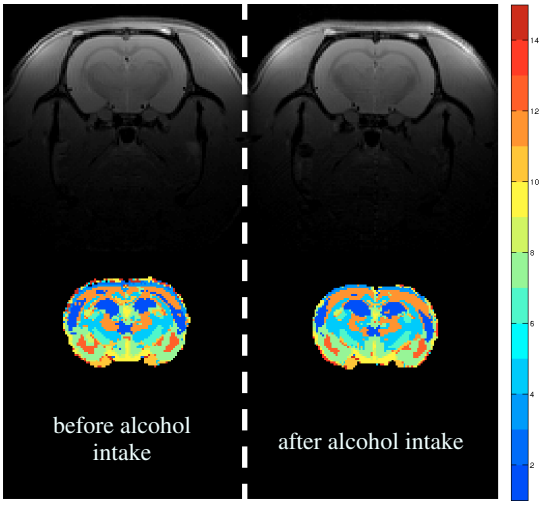

Figure 3. Map of labels of a subject before (left column) and after (right column) alcohol intake.

IV. Conclusion

The hard-alcohol consumption effect in the brain can be studied using the microstructural information of tissues derived from MRI acquisition.

In this work, a fuzzy tissue classification employing three microstructural parameters (T1 and T2 relaxation times and fractional anisotropy) widely used in the study of mental diseases is proposed. The distribution of quantitative parameters derived from MRI has been modeled by a finite weighted sum of multivariate Gaussian distribution where each component of the model represents a particular tissue in the brain.

The non-trivial problem of defining the number of clusters has been solved using the BIC value. Once the optimal number of components of the FGM has been calculated, the structure of covariance matrix of the model has been studied obtaining that the data are better classified if an unconstrained matrix is used. This conclusion is in accordance with other results that propose that partial volume effect due to the resolution of MR images can be modeled by non-diagonal matrix. After the model is defined, all voxels in the brain are classified using the largest posterior probability of belonging to each component obtaining a similar distribution of the clusters within the brain in all the cases.

Using microstructural information a Linear Discriminant classifier is implemented with the aim to identify subjects who have been exposed to alcohol and validated using a leave-one-out strategy. The 93.75 % of subjects are correctly classified suggesting that the study of quantitative parameters derived from MRI are an important tool in the identification of areas related to hard alcohol-consumption effect in the brain.

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