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Optimisation of statistical methodologies for a better diagnosis of neurological and psychiatric disorders by means of SPECT

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Abstract

In the last years there has been a wide consensus on the importance of brain imaging in assessing neurodegenerative and psychiatric disorders. Different techniques for functional and anatomical examination are currently clinically implemented in neurology and psychiatry to improve sensitivity, specificity and accuracy of the diagnosis of various diseases. In addition, the increasing life expectancy in the Western world raises the social importance and the economical impact of age-related neurodegenerative disorders since the incidence of Alzheimer disease and Parkinson disease is higher in the elderly.

An early diagnosis of neuro-psychiatric diseases and the assessment of "natural" changes of regional cerebral blood flow (rCBF) distribution during normal aging are hence of utmost importance.

In the recent past brain disorders have extensively been investigated by means of optimised nuclear medicine techniques, instruments and algorithms. Diagnosis can be better achieved by identifying those structures in which CBF or metabolism deviate from normality resulting in significant changes as compared to a reference database.

In the present paper we present some studies investigating, by means of recently implemented diagnostic tools, patients bearer of various neuro-psychiatric disorders. The improved nuclear medicine techniques and instrumentation, the state-of-the-art software for brain imaging standardisation and the use of sophisticated multivariate data analysis are extensively reviewed.

Keywords: SPECT, neuro-psychiatric diseases, standardisation software, multivariate analysis, functional connectivity

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Introduction

The importance of brain imaging in the assessment of cerebrovascular, neurodegenerative and psychiatric disorders steadily increases. Different techniques for functional and anatomical investigations are clinically implemented in neurology and psychiatry in order to improve sensitivity and accuracy. On the other hand, the longer life expectancy in the Western world raises the social importance and the economical impact of age-related disorders.

Alzheimer's disease has a prevalence of approximately 1 percent among those 65 to 69 years of age and increases with age to 40 to 50 percent among persons 95 years of age and over. Parkinson's disease has a prevalence of approximately 0.5 to 1 percent among persons 65 to 69 years of age, rising to 1 to 3 percent among persons 80 years of age and older [1]. The extrapolation of the AD prevalence curve suggests that all individuals would be demented at the age of 113 years.

The neuro-psychiatric and behavioural abnormalities are often source of considerable patient and caregiver distress, while a proper diagnosis also contributes to the decision of institutionalizing these patients. In addition, an early diagnosis by means of functional imaging techniques and a better patient management could result in considerable savings for the community, i.e. 1138 USD per AD correct diagnosis in USA [2].

In this scenario and in the perspective of future treatment pro-

grams it will be more and more important to improve the accuracy at any stage of the methodologies meant to discover the disease, hence anticipating treatment.

The development and improvement of nuclear medicine techniques and the optimisation of instruments and algorithms for functional brain imaging could be applied at various clinical settings as well as in basic neuro-physiological studies. As for neuroimaging a fair diagnosis can be achieved by identifying those structures, irrespectively for the size, in which the cerebral blood flow (CBF) or metabolism modifications deviate from normality resulting in significant changes as compared to a reference database. Comparison of scans in neurological and psychiatric studies is of utmost importance. The diagnostic value of an image is enhanced if the patient's scan can be compared to an average one obtained from a control group.

This review concentrates on the capability of single photon emission computed tomography (SPECT) and CBF to detect fine functional and pathological changes. This can be accomplished by the implementation of high resolution SPECT, by new software able to automatically standardize the brain space and data across scans and through the use of advanced statistical methods. The examples of implementation in research are extracted from papers and recent communications at conference of our group and will be shortly commented.

Methodological considerations

The assessment of CBF patterns in various brain disorders by SPECT or positron emission tomography (PET) have in the past mainly been carried out either by visual evaluation [3–6] or by outlining the regions of interest (ROIs) in a manual or semiautomatic mode [7–10]. Results were obtained by computing ratios between target and reference regions.

Such methods are time-consuming and poorly reproducible, might suffer from excessive operator's influence in the choice of the ROIs and, due to the variable shape of human brains, lack of spatial normalization, resulting in anatomical in-homogeneous brain samples among subjects.

In the recent past the most advanced research groups across the world dealing with neuroimaging have been implementing a semi-automatic approach for brain regions identification that could be in a second step used clinically to assess CBF changes in a single individual as compared to a population of patients and normal controls.

The process of transforming the images into a common three--dimensional (3D) coordinate space, where each space element (voxel) corresponds to the same anatomical entity in all images under consideration, is denoted as spatial standardization. By carefully standardizing each scan to an age-related rCBF data--base of control subjects it is possible, by means of subtractions images and/or statistical comparisons, to precisely identify regions with abnormal flow. This opens up the conditions for a new approach in research and will allow an easier and earlier diagnosis. The 3D analysis, as compared to conventional 2D data representation on transversal slices, is less dependent on errors in outlining position. Furthermore, the 3D VOIs analysis reduces the variance due to counting statistics since the number of voxels in a functional region (VOI) is larger than a number of pixels in a 2D ROI.

The advantage of this technique is the possibility to fully exploit the knowledge of the rCBF pattern as assessed in a group of normal subjects by using it as a reference for patients groups studies.

In clinical routine, it is possible to compare each single patient to a group of several age matched normal subjects. Control subjects are grouped according to their age, the uptake in the standardized volume is averaged and a reference image containing the rCBF information for all subjects is created. To highlight the possible pathology in the most of the cases the patient image is subtracted from the reference one (Figure 1).

In some cases, especially in psychiatric disorders, the pathological status results in an increased rCBF. In this case subtracting the reference image from the pathological one will highlight the changes.

In 3D analysis, the inclusion of the white matter makes the

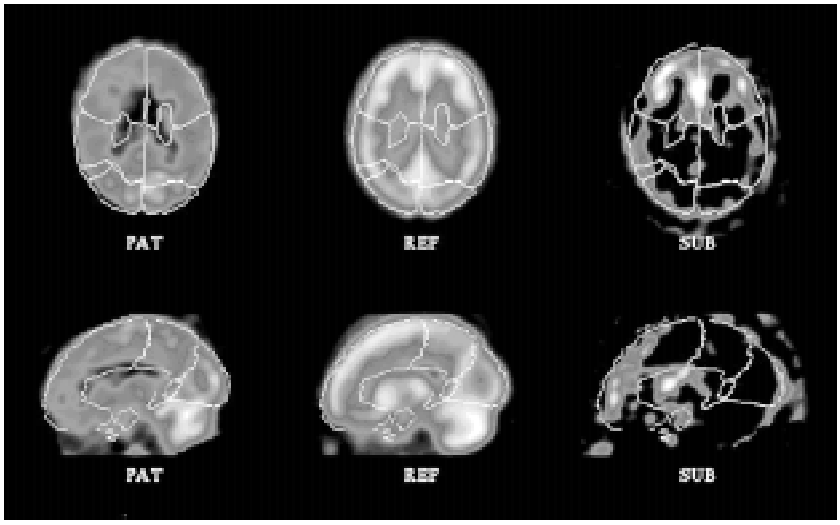

Figure 1. Inter-subject comparison. 76-year-old man, with symptoms of dementia since 10 yr admitted to the hospital for syncope attacks. The SPECT scan is consistent with a diagnosis of Frontal Lobe Dementia. The decreased tracer uptake in frontal lobes is highlighted by the subtraction image (SUB), obtained by subtracting the patient's image (PAT) from the reference image (REF). This latter is the average of 12 age matched controls. The two sets of data have been standardised into a common CBA space. The colour scale is in arbitrary units.

sample more representative for global neurodegenerative changes and VOIs can be positioned on both anatomical and functional regions improving the physiological significance of the analysis. White matter is an important part of the neuronal system and it is affected by neurodegenerative, cerebrovascular and psychiatric disorders to the same extent as grey matter. Grey matter perfusion is 2.0–3.5 times higher than white matter perfusion [11–12]. When 3D analysis is performed on extensive data volume the resulting rCBF is then calculated by averaging, in a certain VOI, a variable number of counts detected in both grey and white matter kernels. Performing SPECT rCBF studies using modern software working in 3D, we have to keep in mind that results obtained and comparison with older data collected with more conventional 2D analyses should be weighed considering the differences in methodology, the used radiopharmaceutical and camera system characteristics.

Standardization software

In the recent years, several 3D digitized spatial standardization software have been proposed and some have extensively been used both in research and clinical investigations [13–14].

The most of them share similar principles and can be classified into two categories: the voxel-based ones (i.e. SPM, NEUROSTAT and BRASS) and the ones based on neuroanatomy (i.e. CBA). We will briefly describe the mostly implemented ones.

Statistical parametrical mapping

Statistical parametrical mapping (SPM) [15], is the worldwide mostly used voxel-based standardization software in brain imaging for between- and within-subject CBF comparisons. Images are spatially standardized into a common space, and smoothed. Parametric statistical models are summed, at each voxel, using the General Linear Model to describe the variability in the data. Hypotheses expressed in terms of the model parameters are assessed at each voxel with univariate statistics. This results in an image whose voxel values are statistics, producing t-statistical maps of significant changes in distribution and basing the output on the analyses of clusters of voxels. Such analysis should to take into account the statistical threshold as well as the size of the cluster in relationship to the implemented methodology: the higher the spatial resolution of the camera the smaller the size of cluster of voxels for statistically significance.

NEUROSTAT

NEUROSTAT [16] is a software library for neurological and biomedical image analysis including programs for brain activation studies, group comparison, three-dimensional stereotactic surface projections and co-registration SPECT-MRI-PET. Anatomic standardization is performed by an automated algorithm estimating the location of the bicommissural line on the scan mid-sagittal plane and realigns the image to such orientation between individuals. Regional differences between individual's scan and a standard atlas brain are minimized automatically by linear scaling and nonlinear warping techniques. Original images are then resampled into a stereotactic image format minimizing anatomic variations across subjects [17–19]. For image interpretation NEUROSTAT uses a 3D stereotactic surface projection technique to depict on the brain surface the cortical CBF and metabolic activity.

It also calculates a z-score to identify statistical deviations from a control subjects' database. Stereotactic coordinates will also allow for a precise localisation of signals.

NEUROSTAT output has recently been validated versus the gold standard of SPM in normal subjects yielding very small difference in standardisation when compared to MRI [20–21].

BRASS

BRASS [22–26] is a standardization software that matches, with automatic masking, patient images to 3D reference templates of normal patients. Defects are localized and quantified against a database of normal patients on a voxel-by-voxel basis. The marked voxels can be assessed statistically, using the standard deviation criterion or within a 3-dimensional map of regions-of--interest. BRASS analyses many types of images, including ^{99m}Tc-HMPAO, ^{99m}Tc-ECD, ^{123}I-IBZM dopamine receptor, FDG-PET and recently FP-CIT (DAT) images.

During the last years several research groups have collected both patients and controls scans acquired with different modalities. At the moment being BRASS is able to provide 19 FDG templates from 4 PET scanners from 6 centres, 15 perfusion SPECT templates acquired with 2 tracers and with 6 different cameras in 8 centres and 18 Dopamine Transporters templates acquired with 4 tracers and 5 different cameras in 5 centres.

In total 52 Databases are available for BRASS users in order to match their patients data to a larger control group. The data--base availability works towards a more widespread collaboration among centres in order to save money and energies and avoids to build expensive in-house healthy subjects database. The drawback of such strategy are the in-homogeneities of the implemented cameras and of the scanning and radiopharmaceutical protocols in the different centres that should be corrected by careful phantom studies and by the implementation of a strict and homogenous patients management [25].

Computerised brain atlas

The computerised brain atlas (CBA) is a software tool originally developed by Greitz et al. [27], and applicable to any brain imaging modality. It is based on data from one cryosectioned brain in combination with information from the literature. It contains 3D surface descriptions (VOIs) of approximately 400 brain structures including the brain surface, the ventricular system, the cortical gyri and sulci, as well as the cortical cytoarchitectonic areas (Brodmann areas). The major basal ganglia and the brain stem nuclei are also included (Figure 2).

All image sets are spatially normalized into the stereotactic space of the atlas by using the global polynomial transformation [28]. It consists of translations, rotations and linear scaling along and around each of the three image axes. It also contains 18 non--linear shape-deforming parameters, which makes it possible to individualize the shape of the brain. The fully automatic method is generally used, in which all scans are registered to a SPECT template [29]. Computerised brain atlas identifies the brain surface,

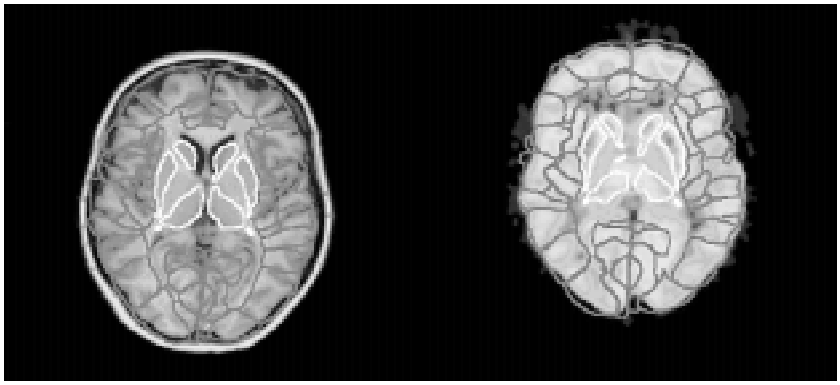

Figure 2. Computed brain atlas template fit to an MRI (left) and to a SPECT study before spatial standardization. All cortical brodmann areas and central structures are defined by the atlas volumes of interest.

the ventricular system and some central nuclei and fits to the aforementioned structures by minimizing the difference in voxel intensity. Subsequently, it deforms and stretches these structures, maximising a simple similarity measure, to fit them to a previously defined reference SPECT scan. A major advantage of the technique is that it creates an almost fully automatic tool able to decrease the analysis time and to standardize patients providing additional anatomic information. The segmentation of the brain in VOIs reduces the number of variables to an amount that is possible, in a second step, to submit to multivariate analysis (i.e. PCA).

Single photon emission computed tomography

Modern gamma camera are operating on-line to a computer system for additional signal and image processing, image display and tomographic reconstruction are designed with more than one camera head, typically three. Tomographic examination is a pre-requisite for rCBF single photon emission computed tomography (SPECT), due to the complex brain anatomy with superimposed anatomo–functional structures. The resulting contrast-enhancing effect of the tomographic registration technique is of great importance since the differences between normal and pathological uptake in various brain regions may be rather small.

The study of rCBF using SPECT is based on depicting the distribution of ^{99m}Tc-HMPAO, ^{99m}Tc-ECD or [^{123}I]-Iodoamphetamine, which are imaged in the brain after intravenous administration. Despite that [^{123}I]-Iodoamphetamine may best represent rCBF, [30], this tracer is rarely used due to a high cost and restricted availability. In this paper we will briefly review only studies performed with ^{99m}Tc-HMPAO.

The intracellular retention of ^{99m}Tc-HMPAO in the central nervous system is the effect of a rapid conversion from the lipophilic into the hydrophilic form at the exposure to endogenous intracellular glutathione, which is a powerful reducing agent. Trapped in the cell, ^{99m}Tc-HMPAO remains practically unchanged being its detection limited by decay of ^{99m}Tc ($t_{1/2}$ = 6.02 h). This specific property allows for scans performed several hours later to still depict the rCBF at the moment of administration [31] and is of paramount importance when examining physically and mentally impaired patients allowing for the administration to be made in a quite environment and the scan postponed according to patients physical and psychological status.

Statistical approach

Univariate analysis

In univariate analysis there is only one variable under consideration. It can be independent, as in the case of CBF data or dependent as in the case of the same subject measured at two different times. In both cases it is possible describe the data in terms of mean and variance (the two parameters of the normal distribution). After testing the two means, possible significant differences need to be explained. The standard approach is to assume that the difference is due to an experimental effect and sources of variance are under control. However, this is not so obvious in neuro-functional studies in which many sources of variance are present. Hence, if we want to study the relationships among those sources, multivariate analysis has to be implemented.

The *t*-test for dependent samples is the most commonly used method to evaluate the differences in means between two groups of observation made on the same sample of subjects who were tested twice. When group of observation are made on different subjects a *t*-test for independent samples is used. One-way ANOVA is performed when groups are three or more. In such cases nothing can be done about the variation due to individual differences since it is not possible to identify, or subtract, such differences. This is why the *t*-test for independent samples is always less sensitive.

Multivariate analysis

Multivariate statistic provides a simultaneous analysis of multiple independent (i.e sex, disease) and dependent (i.e. Hemispheres, VOI) variables in order to determine the relationship among them. Such statistical approach also introduces regional analyses based on the assumption that correlated patterns exist among different brain regions and such relationships affect reciprocally the investigated variable. Variables may be correlated with each other, and their statistical dependence is often taken into account when analyzing such data. In fact, the consideration of

statistical dependence and intercorrelations between variables make multivariate analysis somewhat different in approach and considerably more complex than the corresponding univariate analysis, in which there is only one variable under consideration. In the multivariate perspective each voxel is considered conjointly with explicit reference to the interactions among brain regions rendering it particularly appropriate for brain studies and providing a complementary characterization of CBF patterns.

Multivariate analysis requires the number of observations (scans) to be greater than the number of components of the multivariate observation (variables, i.e. voxels). In neuroimaging techniques (in which the raw images contain and extremely high number of voxels) the number of variables needs therefore to be reduced, by using ROIs, VOIs or factorial groupings.

It takes into account the statistical inference about the response of the entire brain, without regional specificity. If interactions are present one can move from an "omnibus" effect to regional changes with the limitation of the sample size (ROI/VOI/factor).

Multivariate analysis of variance (MANOVA)

It is an extension of ANOVA methods and it is used when one's design involves one or more categorical independent variables (i.e. groups) and two or more continuous dependent variables (i.e. CBF). As well as identifying whether changes in the independent variables have a significant effect on the dependent variables, the technique also seeks to identify the interactions among the independent variables and the association between dependent variables.

ANOVA and MANOVA could be used with a control variable, called covariate. The control variable is used to test the main and interaction effects of categorical variables (i.e. disease and sex) on one or more continuous dependent variable. For instance, the age of subjects can be used as a covariate to control for initial group differences on blood flow. ANCOVA and MANCOVA are used to control for factors which cannot be randomized but which can be measured on an interval scale.

Controlling for one or more covariate reduces errors and increases the statistical power (sensitivity) of the experimental design.

Discriminant function analysis

Discriminant analysis (DA) is performed at group level to estimate the relationship between groupings performed according to the tested methodology and a gold standard (i.e. SPECT/PET diagnosis *vs.* previous clinical diagnosis). The main objective is to construct rules for assigning future observations to one of the groups in order to minimize the probability of misclassification.

Discriminant analysis individuates which variables are "best" to separate cases into two or more predefined groups. If variables are effective for a set of data, the classification table of correct and incorrect estimates will yield a high percentage correct.

The purposes of DA are: to investigate differences between groups; to determine the most parsimonious way to distinguish between groups; to discard variables which are little related to group distinctions; to classify cases into groups and to test theory by observing whether cases are classified as predicted.

Stepwise DA is the most common application of discriminant function analysis and includes many variables in the study, in order to determine the ones that best discriminate between groups.

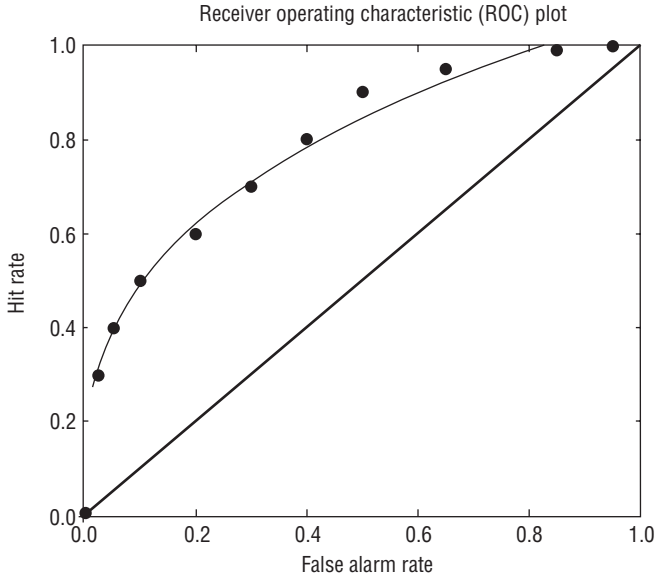

Figure 3. A ROC curve created plotting the true positive rate (Sensitivity) in function of the false positive rate (100-Specificity) for different cut-off points. Each point on the ROC plot represents a sensitivity/specificity pair corresponding to a particular decision threshold. A test with perfect discrimination (no overlap in the two distributions) has a ROC plot that passes through the upper left corner (100% sensitivity, 100% specificity). Therefore the closer the ROC plot is to the upper left corner, the higher the overall accuracy of the test.

Receiver operating characteristic

The ability of a test to discriminate diseased cases from normal cases could also be visually evaluated using receiver operating characteristic (ROC) curve analysis [32–33].

A ROC curve is simply a plot of the true positive (sensitivity) rate against the false positive rate for the different possible cut-off points of a diagnostic test. As would be expected, achieving higher detection performance generally results in an increase in incidents of false alarms: any increase in sensitivity will be accompanied by a decrease in specificity.

This happen because disease and not-disease distribution curves almost always overlap: moving up and down the cut-off points change hits and false positive rates.

In terms of graphical representation, the closer the curve follows the left-hand border and then the top border of the ROC space, the more accurate the test. On the other hand, the closer the curve comes to the 45-degree diagonal of the ROC space, the less accurate the test. The area under the curve is a measure of test accuracy (Figure 3).

K-means clustering

K-means clustering is implemented to create k groups of individuals based on raw data. It splits a set of objects into a selected number of groups by maximizing between variation relative to within variation. K-means is an iterative procedure that assigns cases to a specified number of non-overlapping clusters. The procedure iterates through the data until it successfully clusters your cases. Chi-square is used to test the distribution differences between the obtained clusters and the clinical diagnosis and type.

K-means clustering searches for the best way to separate subjects into different groups. Because it focuses on reducing within-groups sums of squares, k-means clustering is like a multivariate analysis of variance in which the groups are not known in advance.

Principal component analysis

Principal component analisys (PCA) is a data driven technique (i.e., there is no a-priori model or hypothesis) that transforms a number of (possibly) correlated variables into a (smaller) number of not-correlated factors, called principal components. Principal component analysis is totally data-led and is independent by any model or *a-priori* hypothesis.

It does not create effects that are not present in the data, nor does lose information. The first principal component accounts for the highest percentage of the variability in the data and each of the following component accounts for a portion of the remaining variability in a descending scale. The number of factors to be extracted is determined after examining the eigenvalue [34]. This statistical approach introduces regional analyses based on the assumption that correlated patterns exist among different brain regions and such relationships affect reciprocally the rCBF or the metabolism. In PCA each component is orthogonal and functionally not correlated to the remaining ones. It sorts subject-region interaction and it guarantees that regional coupling has been accounted for.

An advantage with a CBA-VOI based approach is that it allows for the investigation of the rCBF relationships between anatomically distributed but physiologically correlated brain regions using PCA. Applying PCA to the VOIs allows for a reduction of the number of variables through the grouping of VOIs into factors. This latter characteristic of PCA might be of utmost importance in analysing pathological conditions in which functionally integrated pathways are involved in the disease process.

Functional connectivity

Functional connectivity implies that pool activities of brain areas "go up and down" together and regions share a significant number of neurons whose dynamic interactions occur at the same time. Correlated areas will have correlated perfusion and neuronal activity. Functional connectivity is simply a statement about the observed correlations and characterizes distributed brain systems.

The functional role played by any component (neuron) of a connected system (brain) is largely defined by its connections. Extrinsic connections between cortical areas are not continuous but occur in patches or clusters (functional segregation, in which cells with common functional properties are thought to be grouped together).

On the other hand functional integration is mediated by the interactions between functionally segregated areas resulting in a general functional connectivity effect on the brain. Functional connectivity characterizes distributed brain systems and implies "model-free" temporal correlations between neurophysiological events: correlated areas will have correlated perfusion and neuronal activity.

The issues related to functional segregation are generally investigated by means of univariate analysis while functional integration is better analyzed by multivariate analysis.

Statistical parametric mapping is typically predicated by functional segregation and analyses regionally specific aspects of functional organization. Principal component analysis and multivariate analysis are inspired by functional integration mediated by anatomical, functional and effective connections that form the basis for characterizing patterns of correlations and describe distributed changes in terms of systems.

Implementation in research

In the recent past standardization software and novel statistical methodologies have been implemented by several groups in both neurodegenerative and psychiatric research in order, by means of the new methodologies, to improve the diagnostic accuracy of functional neuroimaging.

In neurodegenerative research AD has been the most investigated pathology and rCBF distribution pattern has reached a wide consensus among researcher and clinical investigators. In typical cases, it shows reduction of rCBF and metabolism as depicted by SPECT and PET respectively.

The characteristic finding in AD is a bilateral flow reduction in the temporo-parietal cortex [3, 5–7, 9–10, 35–42] and reduced frontal lobe perfusion in the late phase of the disease [43–48]. Such deficits may vary across studies and the reported incidence of the typical rCBF pattern ranges between 30 to 100%, for more advanced stages [3, 49]. In general, such studies have preferably been focused on the tracer distribution in cortical brain tissues. The changes caused by AD on anterior [50–51] and posterior [52] cingulate cortex and on central structures [37, 52–54] have seldom been studied. This latter regions have recently been highlighted by a study in which 3D standardization software and multivariate statistic have been implemented [55] (Figure 4).

Principal component analysis when applied by means of CBA to 54 VOIs covering the most of the cortex and deep grey structures resulted in a larger significance map as compared to SPM in discriminating early AD from normal controls [56] (Figure 5).

In a recent paper, Mosconi [57] reviewed the hypothesis that cortical hypometabolism in AD may be the consequence of reduced projections from dysfunctional neurons in distant brain regions suggesting that the hypometabolism found in AD may partly result from neuroanatomical disconnection with the rhinal cortex.

These findings are of utmost importance since they underscore the functional connections between different brain areas in AD and demonstrate that multivariate statistics (i.e. PCA) might increase the depth and the physiological significance of the analysis as compared to univariate analysis (i.e. SPM).

The recent literature about probable AD [5, 58–64] reported an accuracy for SPECT scans performed with ^{99m}Tc-HMPAO between 64% and 95%. Sensitivity varied between 46% and 100% and specificity ranged between 52% and 85%. As for possible AD, sensitivity and specificity decreased to about 84% and 52% respectively [6]. All such studies based on ROI/VOI analysis were designed on the use of the mean count-density/voxel data.

In a systematic review of the diagnostic accuracy of 48 ^{99m}Tc-HMPAO SPECT studies, Dougall et al [65] concluded that clinical criteria may be more sensitive in detecting AD than brain SPECT (81% *vs.* 74%) but this latter is superior in differentiating AD from other types of dementia (91% *vs.* 70%). On the other hand, a re-

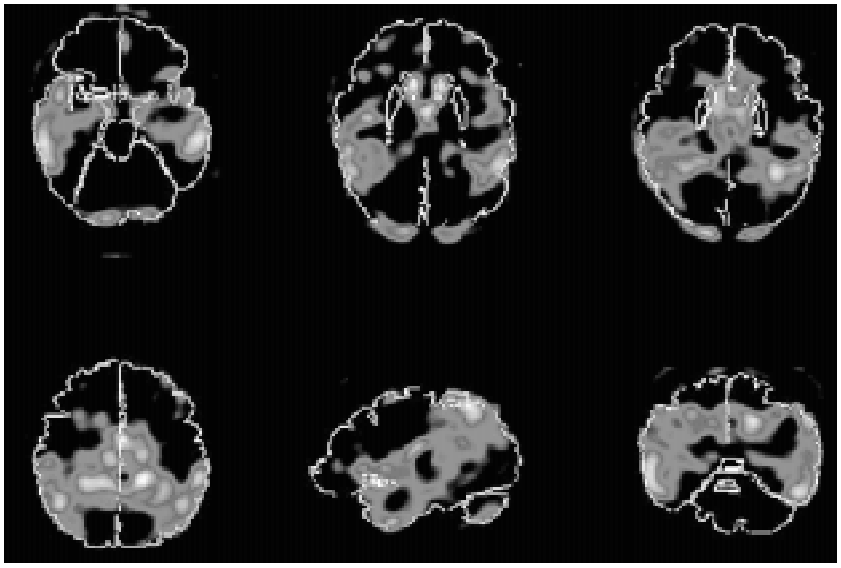

Figure 4. Images obtained subtracting the average data pool of Alzheimer's Disease patients from a group of 19 normal controls. The images highlight decreased CBF in tempo-parietal cortex and in the nuclei caudati, bilaterally. The colour scale is in arbitrary units.

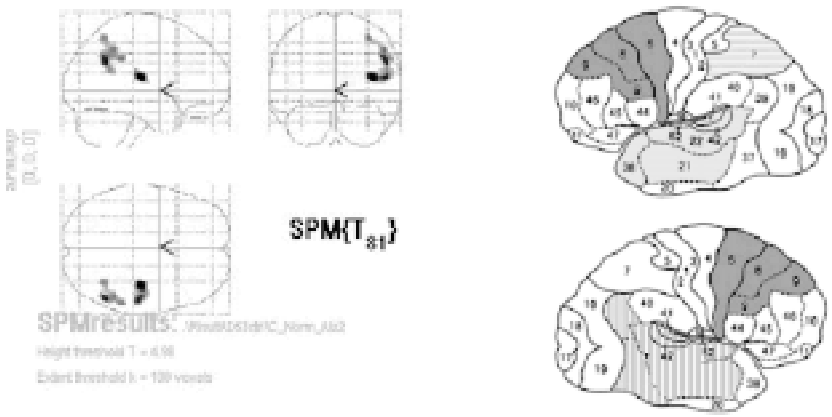

Figure 5. Images showing significant rCBF differences between a control group of 30 individuals and an early Alzheimer's disease group of 27 patients. On the left univariate *t*-statistic maps as depicted by SPM. On the right left (upper) and right (lower) representations of principal components as identified by PCA from a pool of 54 VOIs.

cent FDG-PET study showed provided a sensitivity for distinction between mild to moderate AD and normal controls of 93% [66].

However, applying in mild to moderate AD novel methodological approaches analysing raw data either by k-means clustering and five different intrinsic properties of the image [67] or by means of PCA [56] even with ^{99m}Tc-HMPAO SPECT accuracies of 98% and 90%, respectively were reached.

Functional connectivity has also been recently explored by PCA in Parkinson's disease [68], leading to very promising results.

A relatively new field of application in which functional neuroimaging is gaining more and more consensus is psychiatry. Even if rCBF distribution pattern in the most of psychiatric disorders has not been defined yet, many researches are involved in functional studies on depression, schizophrenia and other psychiatric diseases.

Major depressive disorder (MDD), a primary idiopathic disease characterised by the occurrence of depressive episodes (unipolar depression), is a prevalent clinical condition affecting 26% of the population on a life-time basis [69] and in the next years, also due to the western world life-style, will become one of the dominant neuro-psychiatric and social issues.

A recent paper reviewing 11 studies with 218 unmedicated patients with depression found that the decreased ^{99m}Tc-HMPAO distribution in frontal regions compared to control subjects was the most common finding [70]. Increased ^{99m}Tc-HMPAO distribution in various regions, including frontal regions, was also reported in a few studies [71–79]. It is yet not clear if alterations of tracer distribution reflect 'trait' phenomena that are present prior to onset of overt depression, or 'state' phenomena. It is also un-

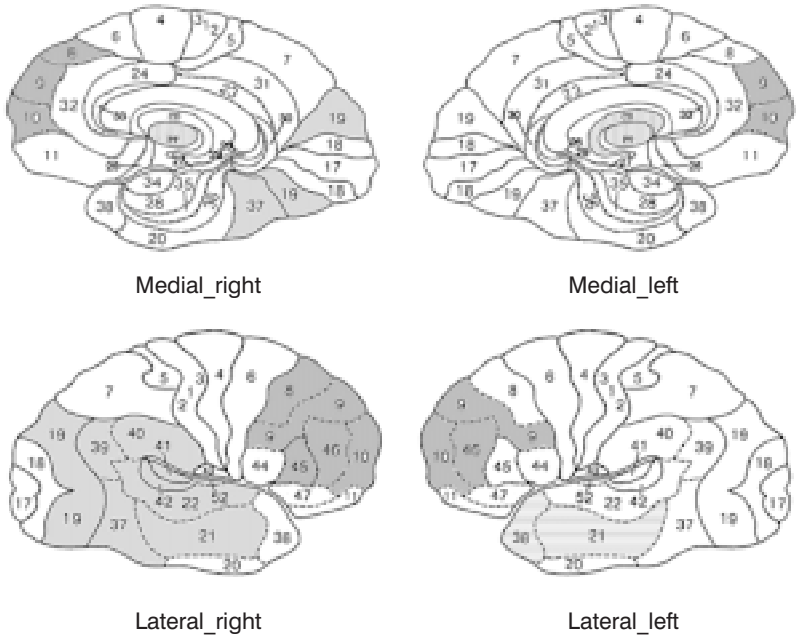

Figure 6. Graphical representation of lateral and medial views of hemispheres depicting four functional regions in which there is a significant rCBF difference between 66 normal controls and 70 MDD patients.

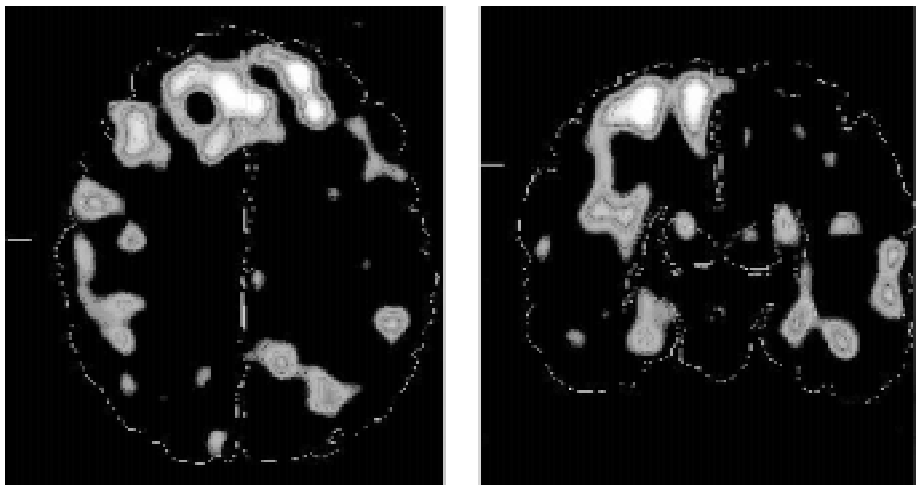

Figure 7. Subtraction image depicting the brain regions in which symptomatic PTSD patients showed higher rCBF response to a script-driven stimulus as compared to non-symptomatic subjects.

known if abnormalities of tracer distribution are pathophysiologically involved in the evolution of depression, or are an additional expression of an as yet unknown etiological factor of the disease. For all these above mentioned reasons the deeper investigation level allowed by multivariate analysis might help in clarifying in MDD the fine and hidden relationships between regions not detectable with conventional statistical analysis.

Applying PCA to patients with major depressive disorder, increased rCBF was found in 4 principal components including regions sharing close anatomical and functional relationships [80––81 (Figure 6) confirming the importance of multivariate analysis in detecting functional changes in pathological conditions.

Multivariate statistics was also recently implemented in a study including, so far, the largest series of subjects investigated in post traumatic stress disorder (PTSD) [82]. In this case ANCOVA, allowed to highlight the entire right hemisphere as the region mostly involved in the emotional response to a script-driven stimulus (Figure 7).

Conclusions

Standardisation software and the creation of control groups add diagnostic value to functional neuroimages. The use of multivariate statistics renders the analysis more complete and able to

properly assess all variables. The application of the proposed methods could give an additional value to functional studies in which visual and semi-quantitative evaluations have not, at the time being, achieved a fair accuracy.

In the field of brain imaging, this the case of psychiatric disorders or mild cognitive impairment in which clear anatomo-pathological findings have not been demonstrate to parallel the clinical status in all circumstances yet.

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