

Analysing brain activity using Statistical Parametric Mapping

P. Nijland



Advisor:
dr. J.B.T.M. Roerdink

June, 1999

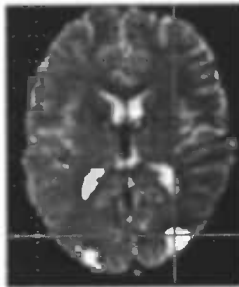
Rijksuniversiteit Groningen
Bibliotheek
Wiskunde / Informatica / Rekencentrum
Lindendreef 5
Postbus 800
9700 AV Groningen

Analysing brain activity using Statistical Parametric Mapping

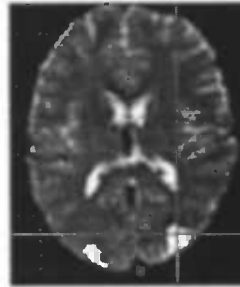
P. Nijland

June 1, 1999

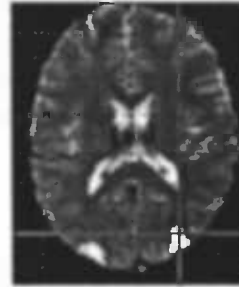
$z = 10\text{mm}$



$z = 12\text{mm}$



$z = 14\text{mm}$



Abstract

The purpose of this thesis is to get an insight in the SPM method, which stands for statistical parametric mapping. SPM is a widely used technique, as well as a software package, for detecting and visualising brain activation. The purpose of this thesis is to find out how SPM works at a theoretical as well as at a practical level.

The thesis begins by some theory about different scanning techniques of the brain and how brain activation can be detected. Different approaches for doing analysis are discussed.

Before doing statistical analysis different steps have to be carried out. These steps include registration of brain scans, mapping brain scans to a template and smoothing of brain scans. A theoretical background of these steps is given to get a deeper insight in SPM. The statistical analysis is discussed in detail afterwards. The practical use of SPM involves using datasets, where one or more subjects are scanned while performing one or more tasks. In this thesis 4 fMRI datasets were used of a subject performing a memory task. Significant brain activation can be seen in the visual cortex of the brain. Finally, AFNI is used to compare the results of SPM with the results of an independent program.

Preface

I would like to thank the people who helped me with my graduation assignment. Especially, I would like to thank Dr. J.B.T.M. Roerdink, for accompanying me with a lot of time and effort, R.P. Maguire, for getting me started and for giving me an account on the GNIP-server and F.W. Cornelissen for providing the data and giving me some feedback on the data.

Contents

| | | |
|----------|--|-----------|
| 1 | Introduction | 5 |
| 2 | Basic concepts of brain activation studies | 6 |
| 2.1 | PET and fMRI | 6 |
| 2.2 | Principles of functional organization | 7 |
| 2.2.1 | Functional integration or functional segregation | 7 |
| 2.2.2 | Approaches for doing analysis | 8 |
| 2.2.3 | Functional connectivity and effective connectivity | 8 |
| 2.2.4 | Spatio-temporal scales | 9 |
| 2.2.5 | Multivariate techniques | 10 |
| 2.3 | Statistical parametric mapping | 10 |
| 2.3.1 | Implementation | 11 |
| 2.4 | Required stages for data analysis | 13 |
| 2.4.1 | Realignment | 13 |
| 2.4.2 | Spatial normalisation | 13 |
| 2.4.3 | Spatial smoothing | 13 |
| 2.4.4 | Statistics | 14 |
| 3 | Spatial transformation of images | 15 |
| 3.1 | Resampling images | 16 |
| 3.2 | Smoothing | 17 |
| 3.3 | Transformations | 18 |
| 3.4 | Optimisation | 19 |
| 3.5 | Intensity transformations | 20 |
| 3.6 | Spatial Normalisation | 21 |
| 4 | Statistical analysis | 22 |
| 4.1 | The general linear model | 22 |
| 4.2 | Statistical inference | 23 |
| 4.2.1 | Contrasts | 25 |
| 4.3 | Spatial extent as additional tool to test significance of activation | 26 |
| 5 | Analysing the data | 28 |
| 5.1 | The fMRI data | 28 |
| 5.2 | Preprocessing the data | 29 |
| 5.2.1 | Moving the <i>raw</i> data to analyze files | 29 |
| 5.2.2 | Realignment | 30 |
| 5.2.3 | Coregister | 30 |
| 5.2.4 | Normalize | 31 |
| 5.2.5 | Changed defaults for normalisation | 31 |
| 5.2.6 | Smoothing | 32 |

| | | |
|----------|-------------------------------------|-----------|
| 5.3 | The tests | 32 |
| 5.4 | fMRI statistics options | 33 |
| 5.5 | SPM output | 35 |
| 5.6 | Results | 38 |
| 5.6.1 | Detailed results | 45 |
| 5.7 | AFNI in comparison to SPM | 51 |
| 6 | Conclusions | 62 |
| A | Wordlist | 63 |

1 Introduction

There are a lot of programs available for detecting and visualising brain activation. From the medical world there is a big interest in these programs. In cooperation between the AZG (the academic hospital in Groningen, the Netherlands) and the RuG, the GNIP (Groningen Neuro-Imaging Project) was founded. A project of the GNIP was to look at these programs as a first step for the AZG to use one of the programs. The program used in this thesis is SPM, one of the well-known programs of this genre.

SPM uses a method called statistical parametric mapping to show activation on fMRI or PET scans. SPM is developed by the methodology group at the Wellcome Department of Cognitive Neurology under the supervision of Karl Friston. SPM is free to use, there is no manual available, but there are articles about its implementation. The version of SPM used in this thesis, SPM96, is available at:

<http://www.fil.ion.ucl.ac.uk/spm/>

In this thesis the SPM-program is used to find the parts of the brain which are activated when a subject is performing some sorts of memory tasks. The memory tasks are divided in a task of remembering a single visual presented character and a task of remembering colour-, and brightness patterns.

A comparison is made between the results of SPM and the results of AFNI [6]. AFNI is another program which can be used to detect brain activation and is used in this thesis for a comparison with SPM.

The provided data is fMRI data from the AZG (the academic hospital in Groningen, the Netherlands).

2 Basic concepts of brain activation studies

In the first half of this section a comparison is made between PET and fMRI. In the remaining part of this section we consider the conceptual and mathematical models that are commonly employed in analysing functional data. This step is further divided in:

Principles of functional organization The Principles of brain organization and their relation to the analytic techniques which are available are discussed. A theoretical distinction is made between *functional integration* and *functional segregation* and a corresponding distinction between *multivariate* and *univariate* analyses at a procedural level. Eigenimage analysis, multidimensional scaling, multivariate analysis of covariance (ManCova) and canonical variates analysis (CVA) are all part of multivariate analyses. The univariate approaches collectively fall under the heading of statistical parametric mapping.

Statistical parametric mapping This is the analysis used by SPM. The section explains how statistical parametric mapping (SPM) works and the advantages over multivariate approaches are explained.

Required stages for data analysis These are the stages of data analysis that are required to implement the analyses, mentioned above. These stages include realignment, spatial normalization, smoothing, statistical analysis and, when appropriate, statistical inference.

2.1 PET and fMRI

From the early 80s positron emission tomography (PET) has had a dominating position in functional anatomy. Just recently functional magnetic resonance imaging (fMRI) has developed into an alternative and powerful technique. PET measures blood flow on a spatial scale of about 6mm and a temporal scale of approximately 30 seconds. fMRI is sensitive to the oxygenation of blood and has a spatio-temporal scale of about 1-3mm and one or more seconds. The effective resolution of fMRI is limited by the spatio-temporal organization of **hemodynamic** responses of the brain (2-5mm and 5-8 seconds). Local increases in neural activity cause both a relative deoxygenation of blood and an increase in perfusion of the blood, that quickly reverses the deoxygenation, leading to an increase in oxygenation for several seconds. PET measures changes in blood flow or perfusion in terms of the amount of radio labelled water that accumulates locally but this takes several tens of seconds to a minute or so.

Due to the relatively long half-life of the tracers used, PET can only measure

responses summed over fairly long periods of time. This puts some fundamental constraints on its experimental designs. PET can only be used to measure differences in brain states. fMRI on the other hand can be used in two ways. The response to epochs of repeated stimuli or to continuous task performance can be measured, or alternatively just like PET the response to a single stimulus can be measured.

2.2 Principles of functional organization

2.2.1 Functional integration or functional segregation

There appear to be two fundamental principles of functional organization in the brain; *functional integration* and *functional segregation*, with the integration within and among functionally segregated areas produced by functional or effective connectivity. Functional segregation, integration and connectivity are important areas of exploration in neuroscience. Functional imaging is an example of an area of neuroscience where the exploration of these three areas is an important endeavour.

The distinction between functional segregation and integration can be reduced to the distinction between *localisationism* and *[dis]connectionism* that dominated thinking about **cortical** function in the nineteenth century. Since the early anatomic theories of Gall, the identification of a particular brain region with a specific function has become a central theme in neuroscience. However functional localization was not easy to demonstrate. For example, a meeting that took place on the morning of August 4th 1881, entitled 'Localisation of function in the **cerebral cortex**' showed the difficulties of attributing function to a **cortical** area, given the dependence of **cerebral** activity on underlying connections [24]. Goltz considered the **excitation** method inconclusive, in that movements elicited might have originated in related areas, or could have spread to distant areas. Summarized the **excitation** method could not be used to infer functional localisation because localisationism discounted interactions, or functional integration among different brain areas. An observation on patients with brain lesions [1] some years later demonstrated *disconnection syndromes* and the rejection of localisationism as a complete or sufficient explanation of **cortical** organization was a fact.

Functional localisation implies that a function can be localised in a **cortical** area, segregation on the other hand suggests that a **cortical** area is specialised for some aspects of perceptual or motor processing, and that specialisation is anatomically segregated in the **cortex**. The **cortical** infrastructure of a single function may then involve many specialised areas which are united, due to their functional integration among them.

Despite advances over the past century, the central issue remains unresolved. Are the **physiological** changes caused by some sensorimotor or cognitive challenge best described by activation of the functionally segregated areas or are they better described in terms of the behaviour of anatomically distributed, but functionally integrated systems. A simple solution is to acknowledge that both perspectives are valid and that research about brain activations can proceed at both levels.

2.2.2 Approaches for doing analysis

There are two basic approaches when analyzing and interpreting brain activation. These approaches directly depend on the distinction between functional integration and segregation, discussed above. The first set of approaches is based on correlated **physiological** dynamics in different parts of the brain and deals with distributed brain systems. These approaches are multivariate in nature. The second set depends on detecting focal differences using a functional segregation or specialisation view of the brain.

The first set of approaches to analysis includes *eigenimage analysis* and *multidimensional scaling*. These are mathematically closely related, but in relation to functional imaging data they have different interpretations. A disadvantage of these two methods is that neither eigenimage analysis nor multidimensional scaling can be used to make statistical inferences about what is observed (i.e. estimate the significance of the results obtained in terms of a p value). Another method which is part of functional integration is multivariate analysis of (co)variance (*ManCova*) with canonical variates analysis (CVA). Like eigenimage analysis CVA characterizes brain responses in terms of anatomically distributed profiles or *spatial modes*. In contrast to the other techniques *ManCova* does provide for statistical inference about the responses, but this inference is about the entire brain. When looking for regionally specific effects one turns, finally, to *statistical parametric mapping*. Statistical parametric mapping is part of functional segregation or specialisation and is used to characterize **physiology** in terms of regionally specific responses. This is done by treating each voxel separately in a univariate way. By performing voxel-wise statistical analyses after that in parallel, an image of statistic or 'significance' is created.

2.2.3 Functional connectivity and effective connectivity

Interaction between different brain areas can be characterized by functional or effective connectivity. Functional connectivity has been defined as the *temporal*

correlations between spatially remote neurophysiological events[11]. Effective connectivity on the other hand is defined as *the influence one neural system exerts over another*[10]. Functional connectivity is simply a statement about the observed correlations; it does not provide an insight in how these correlations have originated. To examine the integration within a distributed system, defined by functional connectivity, one turns to *effective connectivity*. In **electrophysiology** a close relationship exists between effective connectivity and synaptic efficacy: "It is useful to describe the effective connectivity with a connectivity matrix of effective synaptic weights" [18]. Another description: "the [**electrophysiological**] notion of effective connectivity should be understood as the experiment and time dependent, simplest possible circuit diagram that would replicate the observed timing relationships between the recorded neurons" [3].

2.2.4 Spatio-temporal scales

Although functional and effective connectivity can be used at a conceptual level in both neuroimaging and **electrophysiology** they differ fundamentally at a practical level. This is because the time-scales and nature of the **neurophysiological** measurements are very different (seconds vs. milliseconds and hemodynamic vs. spike trains).

Clearly there is an enormous difference between correlations in regional **cerebral** blood flow (rCBF)¹ measured over seconds and the fast neural interactions on a time-scale of milliseconds. Functional connectivity is not necessarily due to effective connectivity, but there is reason to believe that there is a relationship between functional connectivity at a large-scale **physiological** level and at a neural level. If two areas share a functional connectivity then their pool activity will go up and down together. A relationship between functional and effective connectivity will imply that two regions with high pool activity will share a significant number of neurons whose dynamic interactions occur within a time frame of milliseconds. It is suggested that this is the case. There are two lines of evidence in support of a relationship between fast dynamic interactions and slower variations in pool activity: (i) Aertsen & Preissl [3] have investigated the behaviour of artificial networks, analytically and using simulations. They concluded that *short term effective connectivity* varies strongly with, or is modulated by, *pool activity*. Pool activity was defined as the product of the number of neurons and their mean firing rate. (ii) The second line of evidence is experimental and demonstrates that the presence of fast interactions is associated with intermediate or long term correlations between distant neurons. Nelson et al [22] have characterized effective connections between neurons, or small groups of neurons,

¹regional **cerebral** blood flow

in some parts of the brain. The idea is in summary that co-activated regions will have increased (correlated) perfusion and neuronal pool activity.

2.2.5 Multivariate techniques

Eigenimage analysis The characterization of distributed brain systems is a powerful use of functional connectivity. Patterns of correlations in the brain are more interesting than the functional connectivity between two points, these patterns can be identified as follows. Principal Component Analysis (PCA) or Singular Value Decomposition (SVD) is applied to the functional connectivity (covariance) matrix, which is obtained from a time-series of images. The resulting eigenimages (principal components or spatial modes) each correspond to a spatial pattern or distributed system, with regions taken together by their functional interactions.

Multidimensional scaling In contrast to the method which is often used in neuroimaging, namely that of *mapping function into an anatomical space*, a complementary approach is introduced here, *mapping anatomy into a functional space*. The idea is to construct a space where functional connectivity is used to define the distance between two anatomical areas. The topography, or proximity relationships, in this space embody the functional connectivity among brain regions. The higher the functional connectivity the closer the regions. Multidimensional scaling has a lot in common with eigenimage analysis, because both eigenimage analysis and multidimensional scaling use the same mathematics to characterize anatomically distributed changes in the brain which show **cortico-cortical** interactions in terms of functional connectivity.

ManCova-CVA ManCova uses a linear equation to make inferences about distributed activation effects. These effects can be described using an eigenimage analysis or a CVA. With this multivariate approach all the effects due to activation, confounding effects and error effects are assessed statistically in terms of effects at each voxel and in terms of effects among voxels. The price paid for using a multivariate approach is that one cannot make statistical inferences about regionally specific effects. This is because the inference refers to all the components (voxels) of the multivariate variable (and not to a particular voxel or set of voxels). This is a disadvantage when using multivariate approaches.

2.3 Statistical parametric mapping

The multivariate techniques take explicit account of interactions among brain regions or voxels. In general the effects elicited by experimental design are de-

scribed in terms of spatial modes (i.e. eigenimages or canonical images) that are single, anatomically distributed patterns. The techniques so far are all part of functional integration, we now turn to statistical parametric mapping which falls under functional segregation and where the effects are described in terms of the number of regionally specific changes.

The functional role played by any component (e.g. a neuron) of a connected system (e.g. the brain) is largely defined by its connections. Functional segregation demands that cells with common functional properties be grouped together. This architectural constraint in turn necessitates both convergence and divergence of **cortical** connections. If neurons in a **cortical** area share a common responsiveness to some sensorimotor or cognitive challenge then this functional segregation is also an anatomical one. When a subject performs an appropriate sensorimotor or cognitive process, it should lead to activity changes in, and only in, the area of interest. This is the model upon which the search for regional specific effects is based.

Functional mapping studies are usually analyzed with some form of statistical parametric mapping. Statistical parametric mapping refers to the construction of spatially extended statistical processes to test hypotheses about regionally specific effects which is possible because statistical parametric mapping is a univariate approach. Statistical parametric maps (SPMs) are image processes with voxel values that are, under the null hypothesis, distributed according to a known probability density function (usually Gaussian).

The idea behind statistical parametric mapping is simple. One analyzes each and every voxel using any standard (univariate) statistical test. The resulting statistical parameters are assembled into an image (the SPM). SPMs are interpreted as a spatially extended statistical process by referring to the probabilistic behaviour of **stationary** Gaussian fields [2]. **Stationary fields** model both the univariate probabilistic characteristics of an SPM and any stationary spatial covariance structure. 'Unlikely' excursions of the SPM are interpreted as regionally specific effects, caused by some sensorimotor or cognitive process.

2.3.1 Implementation

In this part of SPM the data is modelled in order to partition observed **neurophysiological** responses into components of interest, confounds or components of no interest and an error term. This is done using the general linear model to estimate the components in terms of the parameters associated with the design matrix (see section 4.1).

The contribution of each effect (i.e. each column of the design matrix modelling the various response components) to the observed **physiological** responses is estimated using the general linear model and standard least squares. These estimated contributions are known as parameter estimates. Regionally specific effects are framed in terms of differences among these parameter estimates (e.g. an activation effect) and are specified using linear components or *contrasts*. The significance of each contrast is assessed with a statistic whose distribution has Student's *t* distribution under the null hypothesis. For each contrast or differences in parameter estimates, a *t* statistic is computed (in parallel) for each and every voxel to form a $\text{SPM}\{t\}$. For convenience the $\text{SPM}\{t\}$ is transformed to a Gaussian Field or $\text{SPM}\{Z\}$. Statistical inferences are then made about local excursions of the $\text{SPM}\{Z\}$ above a specified threshold, using distributional approximations from the theory of Gaussian fields. These distributions pertain to simple characterizations of the 'blobs' of activation; namely their maximal value and their spatial extent (or both). The resulting *p*-values can be considered corrected for the volume of brain tested. By specifying different contrasts one can test for a variety of effects. These effects can be divided into three main categories:

Cognitive and sensorimotor subtraction and conjunctions The difference between two tasks can be formulated as a separable cognitive or sensorimotor component. Regionally specific differences in brain activity identify the corresponding functionally specialized area. Cognitive conjunctions can be thought of as a combination of a series of subtractions. If one can identify a series of task pairs whose differences have only a certain component in common, then the region which activates in all the corresponding subtractions can be uniquely associated with the component in question.

Parametric designs The idea behind parametric designs is that regional physiology will vary systematically with the degree of cognitive or sensorimotor processing. An example of such an experiment is the significant correlation between rCBF and the performance of a visually guided motor tracking task, demonstrated by Grafton [20].

Factorial designs In these designs two or more factors are combined in the same experiment. The effect of one factor on the effect of the other, is assessed by the interaction term. **Psychopharmacological** activation studies are examples of factorial design [12]. In these studies subjects perform a series of **baseline-activation** pairs before and after being given a drug. The interaction term reflects the drug effect on the task-dependent activation [21].

2.4 Required stages for data analysis

2.4.1 Realignment

Due to the movement of a subject in a scanner, the data can be distorted. This movement-related variance has to be removed to make it possible to properly analyze the data. For PET the movement, relative to the first scan, is estimated using a least square method that is obtained after linearising the problem using a first order Taylor series. The realignment then simply involves reslicing the scans, using the estimates. fMRI time-series constitute a different case. This is because movement in earlier scans can affect the signal in subsequent scans (due to differential spin-excitation histories). The realignment for fMRI time-series consists of two parts. First the images are realigned, just like the PET-data. Second there is a mathematical adjustment based on a moving average auto-regression model of spin-excitation history effects.

2.4.2 Spatial normalisation

SPM uses the following method to show the results of the analysis in a conventional way. Before the data is analyzed, the data is moved and 'warped' into some idealized or standard brain. An advantage of this method is that this normalisation method facilitates intersubject averaging. The transformation of an image into a standard anatomical space (which usually is the one described in the atlas of Talairach and Tournoux [27]) corresponds to this spatial normalisation. The spatial normalisation consists of mapping the data to a template. The affine mapping transformation can contain up to 12 parameters. If necessary the transformation can be made nonlinear as well. The template is a standard MRI- or PET template which visually best fits the data.

2.4.3 Spatial smoothing

Smoothing has a number of important objectives. In the first place, it generally increases signal relative to noise. This is because noise usually has higher spatial frequencies than the **neurophysiological** effects of interest, which are produced by **hemodynamic** changes that are expressed over spatial scales of several millimetres. The second reason for smoothing is specific to intersubject averaging. It ensures that **hemodynamic** changes from subject to subject are assessed on a spatial scale at which homologies in functional anatomy are typically expressed. In other words it's unlikely that intersubject brain activity will match on a scale of much less than a millimetre. But it is much more likely that it will match on a scale of say 8mm, evidenced by the success of multi-subject PET activation studies. The final reason is that the data conforms more closely to a Gaussian field model after smoothing. This is important, because the statistical inference makes use of the theory of Gaussian fields. With smoothing there is a trade-off

between the degree of smoothing and the spatial resolution to be considered. This trade-off is especially important for fMRI with its high anatomical resolution. The general approach is to use the least possible smoothing, but to have a smoothness being large enough to ensure the correctness of the statistical inference. Note that the use of spatial smoothing is the subject of intensive scientific debate.

2.4.4 Statistics

In this final stage the **neurophysiological** responses are partitioned into components of interest, confounds or components of no interest and an error term. The partitioning involves estimating the components in terms of parameters associated with the design matrix (see section 4.1). The analysis of regionally specific effects uses the general linear model. The general linear model is an equation which expresses observations as a linear combination of expected components along with some error. The general linear model is necessary for doing statistical analysis. With a contrast the general linear model can calculate the contribution or difference among the estimated parameters, by referring to the error variance. This results in a statistical parametric map, the $\text{SPM}\{F\}$ or the $\text{SPM}\{Z\}$ with associated p-values.

3 Spatial transformation of images

For the realignment and the spatial normalisation of images a technique is used for the spatial transformation of images. This transformation is necessary in functional imaging, because the **hemodynamic** responses are small compared to signal changes that can be caused by a moving subject and for an accurate statistical test it is important that the images are aligned as close together as possible. A scanner can eliminate coarse movements, but motion correction is necessary to eliminate the small movements as well. Motion correction can be split up in two parts. In the first place one must determine the parameter values for a rigid body transformation that optimises some criteria for matching an image to a reference image. When the parameter values are determined one can transform the image by resampling according to the determined parameters.

When one wants to use more than one subject to perform a test it is necessary to put the images in some standard space to allow signal averaging across the subjects. A further advantage when using a standard space is that activation can be reported according to their Euclidean coordinates within this standard space. The most commonly used coordinate system within brain imaging is the space described by Talairach & Tournoux (1988) [27]. When normalising to a standard space one begins by matching the brains to a template image using an affine transformation, followed by introducing non-linear deformations described by some smooth basis functions [9]. Since there is no one-to-one mapping of cortical structures between different brains the images are smoothed prior to statistical analysis in a multi-subject study, so that corresponding activations from the different brains are superimposed.

In some cases one wants to registrate images of different modality. An example is when sites of activation of a functional image are superimposed on a high resolution structural image to accurately localise the activation. Registration of images of different modality is performed by optimising a set of parameters describing a rigid body transformation, as in the case of movement correction, but due to the different modalities the matching needs to be more complex. Another use for this registration is to achieve a more accurate normalisation when computing the normalisation from a more detailed structural image. With the structural and functional images in registration, the computed transformations can be applied to the functional images.

In this section resampling, smoothing and transformations will be described as a basis for the co-registration and normalisation of images. Only intra modality co-registration of images will be described, because inter modality co-registration isn't further used in this thesis.

3.1 Resampling images

When the parameter values for the transformation are determined, it is necessary to resample the image in order to apply the spatial transform. Resampling involves determining for each voxel in the transformed image, the corresponding intensity in the original image. To resample between the centres of voxels some sort of interpolation is needed.

The simplest possible interpolation method is *nearest neighbour* which takes the value of the closest neighbouring voxel. The original voxel intensities are preserved, but the resulting image can be quite blocky. A method which has a much less ‘blocky effect’ is *trilinear interpolation* which is slightly slower than nearest neighbour and has the effect of losing some high frequency information from the image.

| | | | | |
|---|---|---|---|---|
| a | b | q | c | d |
| e | f | r | g | h |
| | | u | | |
| i | j | s | k | l |
| m | n | t | o | p |

Assume we want to calculate the value at point u and that there is a regular grid at coordinates (x_a, y_a) to (x_p, y_p) having intensities v_a to v_p (see the figure above). The value at points r and s are first determined as follows (using linear interpolation):

$$v_r = \frac{(x_g - x_r)v_f + (x_r - x_f)v_g}{x_g - x_f}$$

$$v_s = \frac{(x_k - x_s)v_j + (x_s - x_j)v_k}{x_k - x_j}$$

Then v_u is determined by interpolating between v_r and v_s :

$$v_y = \frac{(y_u - y_s)v_r + (y_r - y_u)v_s}{y_r - y_s}$$

This *bilinear* interpolation method can be easily extended to three dimensions.

If we want to use more than the 8 neighbours to estimate the value at a point we can use *polynomial interpolation* in order to fit a smooth function through the points, and then obtain the value of the function at the desired point. (Nearest

neighbour and tri-linear interpolation are simple low order *polynomial interpolations*. v_q can be determined from pixels a to d as follows. First the coefficients (\mathbf{q}) of a polynomial that runs through these points can be obtained by computing:

$$\mathbf{q} = \begin{bmatrix} 1 & 0 & 0 & 0 \\ 1 & (x_b - x_a) & (x_b - x_a)^2 & (x_b - x_a)^3 \\ 1 & (x_c - x_a) & (x_c - x_a)^2 & (x_c - x_a)^3 \\ 1 & (x_d - x_a) & (x_d - x_a)^2 & (x_d - x_a)^3 \end{bmatrix}^{-1} \begin{bmatrix} v_a \\ v_b \\ v_c \\ v_d \end{bmatrix}$$

v_q can now be determined from these coefficients:

$$v_q = (1 - (x_q - x_a)^2 - (x_q - x_a)^3) \mathbf{q}$$

v_u can be calculated in a similar way as v_q . This form of polynomial interpolation is called Lagrange interpolation.

The best interpolation method is to do the interpolation in Fourier space [7]. SPM however uses real space interpolation. The corresponding method to Fourier interpolation in real space is *sinc* interpolation. True sinc interpolation uses every voxel in the image to resample a single point. Speed limitations however require to limit the number of nearest neighbours which can be used. The sinc function extends to infinity, therefore it is truncated by modulating with a window, such as a Hamming window. For one dimension the windowed sinc function using the I nearest neighbours would be:

$$\sum_{i=1}^I v_i \frac{\frac{\sin(\pi d_i)}{\pi d_i} \frac{1}{2} (1 + \cos(2\pi d_i/I))}{\sum_{j=1}^I \frac{\sin(\pi d_j)}{\pi d_j} \frac{1}{2} (1 + \cos(2\pi d_j/I))}$$

where d_i is the distance from the centre of the i th voxel to the point to be sampled, and v_i is the value of the i th voxel. This form of sinc interpolation is preferred by the authors of SPM.

3.2 Smoothing

To reduce the number of local-minima in the optimization part of the realignment algorithm (see section 3.4) smoothing is used. Smoothing is also used before statistical analysis to superimpose activation from different brains, to increase signal relative to noise and to ensure the correctness of the statistical inference. A remark should be made that there is substantial debate in the literature about the validity of this step.

The smoothing used is a discrete convolution with a Gaussian kernel g . The amplitude of a Gaussian at j units away from the centre is defined by:

$$g_j = \frac{e^{-\frac{j^2}{2s^2}}}{\sqrt{2\pi s^2}}$$

where the parameter s is defined by $\frac{FWHM}{\sqrt{8 \ln(2)}}$ and FWHM is the *full width at half maximum* of the Gaussian. The convolution of the function s with g which gives the convolved function t is given by:

$$t_i = \sum_{j=-d}^d s_{(i-j)} g_j$$

This convolution is for one dimension. A 3 dimensional convolution can be accomplished by convolving each dimension in succession.

3.3 Transformations

The general transformation used is the affine transformation. For each point (x_1, x_2, x_3) in an image a mapping can be defined to another point (y_1, y_2, y_3) . In matrix form this mapping M can be formulated as a simple matrix multiplication:

$$\begin{pmatrix} y_1 \\ y_2 \\ y_3 \\ 1 \end{pmatrix} = \begin{pmatrix} m_{11} & m_{12} & m_{13} & m_{14} \\ m_{21} & m_{22} & m_{23} & m_{24} \\ m_{31} & m_{32} & m_{33} & m_{34} \\ 0 & 0 & 0 & 1 \end{pmatrix} \begin{pmatrix} x_1 \\ x_2 \\ x_3 \\ 1 \end{pmatrix}$$

There are also rigid body transformations. These transformations are a subset of the affine transformation and are necessary to co-register images of the same subject together. In three dimensions a rigid body transformation can be defined by six parameters. These parameters are three translations and three rotations about the orthogonal axes. The translation is implemented by the following matrix:

$$\begin{pmatrix} 1 & 0 & 0 & x_{trans} \\ 0 & 1 & 0 & y_{trans} \\ 0 & 0 & 1 & z_{trans} \\ 0 & 0 & 0 & 1 \end{pmatrix}$$

The matrices for the rotations (Θ , Φ and Ω in radians) about the X, Y and Z axes are respectively:

$$\begin{pmatrix} 1 & 0 & 0 & 0 \\ 0 & \cos(\Theta) & \sin(\Theta) & 0 \\ 0 & -\sin(\Theta) & \cos(\Theta) & 0 \\ 0 & 0 & 0 & 1 \end{pmatrix}, \begin{pmatrix} \cos(\Phi) & 0 & \sin(\Phi) & 0 \\ 0 & 1 & 0 & 0 \\ -\sin(\Phi) & 0 & \cos(\Phi) & 0 \\ 0 & 0 & 0 & 1 \end{pmatrix}, \begin{pmatrix} \cos(\Omega) & \sin(\Omega) & 0 & 0 \\ -\sin(\Omega) & \cos(\Omega) & 0 & 0 \\ 0 & 0 & 1 & 0 \\ 0 & 0 & 0 & 1 \end{pmatrix}$$

When registering an image to another image care must be taken about the voxel sizes of the images. In some cases the voxel-sizes of the images will be different. Interpolation can be a solution to this problem, but a simpler solution is to transform the images into a Euclidean space, where measures of distance are expressed in millimetres. For example, if image f is of size $128 \times 128 \times 43$

and has voxels that are $2.1mm \times 2.1mm \times 2.45mm$, the transformation into an Euclidean space is defined by:

$$M_f = \begin{pmatrix} 2.1 & 0 & 0 & -\left(\frac{2.1 \times 128}{2}\right) \\ 0 & 2.1 & 0 & -\left(\frac{2.1 \times 128}{2}\right) \\ 0 & 0 & 2.45 & -\left(\frac{2.45 \times 43}{2}\right) \\ 0 & 0 & 0 & 1 \end{pmatrix}$$

The transformation matrix maps the voxel coordinates to a Euclidean space whose axes are parallel to those of the image. The distance is measured in millimetres, with the origin at the centre of the image.

To co-register, the rigid body transformation has to be determined, which maps the coordinates of image f to the coordinates of image g . This rigid body transformation, which we call M_r , is defined such that $M_g^{-1} M_r M_f$ will register the images. If another image (h) is also in registration to image g in the same manner, then there is also an analogous mapping between f and h .

3.4 Optimisation

When two images need to be registered we have to determine the parameter values for which some function of the parameters is minimised (or maximised). This is called optimisation. An often used optimization is to minimise the sum of squared differences (χ^2) between the two images. The problem is that there are many parameters in the model. These are the parameters that describe the spatial transformation and often nuisance parameters required by the model, such as intensity scaling parameters. Because of the number of parameters it is not possible to search through the whole parameter space. The usual approach is to make an initial parameter estimate, and begin iteratively searching from there. At each iteration, the model is evaluated using the current parameter estimates, and χ^2 is computed. At each iteration a decision has to be made how the parameter estimates have to be modified. The optimisation is stopped when some convergence criterion is achieved (usually when the decrease of χ^2 is smaller than some tolerance level).

The algorithm used by SPM is similar to *Gauss-Newton* optimisation [25] and is as follows. Suppose that $d_i(\mathbf{p})$ is the function describing the difference between the object and template images at voxel i , when the vector with the model parameters has value \mathbf{p} . For each voxel (i), a first order Taylor approximation is used to estimate the value that this difference will take if the parameter vector \mathbf{p} is increased by \mathbf{t} :

$$d_i(\mathbf{p} + \mathbf{t}) = d_i(\mathbf{p}) + t_1 \frac{\partial d_i(\mathbf{p})}{\partial p_1} + t_2 \frac{\partial d_i(\mathbf{p})}{\partial p_2}$$

From this, we can set up a number of simultaneous equations to estimate the values of \mathbf{t} when minimising $\sum_i d_i(\mathbf{p} + \mathbf{t})^2$:

$$\begin{pmatrix} -\frac{\partial d_1(\mathbf{p})}{\partial p_1} & -\frac{\partial d_1(\mathbf{p})}{\partial p_2} & \dots \\ -\frac{\partial d_2(\mathbf{p})}{\partial p_1} & -\frac{\partial d_2(\mathbf{p})}{\partial p_2} & \dots \\ \vdots & \vdots & \ddots \end{pmatrix} \begin{pmatrix} t_1 \\ t_2 \\ \vdots \end{pmatrix} \simeq \begin{pmatrix} d_1(\mathbf{p}) \\ d_2(\mathbf{p}) \\ \vdots \end{pmatrix}$$

For the parameters \mathbf{p} we now can derive an iterative scheme for improving the parameter estimates:

$$\mathbf{p}^{(n+1)} = \mathbf{p}^{(n)} + (A^T A)^{-1} A^T \mathbf{b}$$

where

$$A = \begin{pmatrix} -\frac{\partial d_1(\mathbf{p})}{\partial p_1} & -\frac{\partial d_1(\mathbf{p})}{\partial p_2} & \dots \\ -\frac{\partial d_2(\mathbf{p})}{\partial p_1} & -\frac{\partial d_2(\mathbf{p})}{\partial p_2} & \dots \\ \vdots & \vdots & \ddots \end{pmatrix} \text{ and } \mathbf{b} = \begin{pmatrix} d_1(\mathbf{p}) \\ d_2(\mathbf{p}) \\ \vdots \end{pmatrix}$$

This process can be repeated until χ^2 no longer decreases, or when a fixed number of iterations have been processed. It can happen that the algorithm get caught in a local minimum. To reduce this problem smooth images and good starting estimates should be used. An extra advantage of the use of smooth images is that they make the first order Taylor approximation more accurate for larger displacements. Once the registration is close to the true solution, the registration can continue with less smooth images.

To reduce the required amount of computer memory only a few rows of the matrices A and \mathbf{b} are stored at a time. The calculations can be sped up by calculating the partial derivatives $\frac{\partial d_i(\mathbf{p})}{\partial p_j}$ from the gradients of the images using the chain rule, as described in the next sections.

3.5 Intensity transformations

The images which have to be registered are normally not equal. This means that the optimisation can be assumed to minimise two sets of parameters: those describing spatial transformations and those describing intensity transformations. If there is a linear relationship between the object and the reference (*template*) image then the object image could be matched to a scaled version of the reference image. This is not very often the case: the intensities in one image may not vary linearly with the intensities in the other. For this reason it may be more appropriate to match one image to some function of the other image. For example we could match the object image to a scaled version of the reference image. A polynomial model can also be used for the intensity transformation. More complex variations could be included by modulating with other basis functions

or to use non-linear parameters describing the intensity transformations. It is not necessary to use one reference image. A series of reference images can be used (which all conform to the same space). The idea behind this method is that (ignoring the spatial differences) any given image can be expressed as a linear combination of a set of reference images. In this way reference images can be used with different modalities, different anatomical tissues (e.g. grey matter, white matter) or different anatomical regions (e.g. cortical grey matter or cerebellum). A combination of the above can also be used.

3.6 Spatial Normalisation

All the images are mapped in some standard space when using spatial normalisation (usually Talairach & Tournoux) [27]. Normalisation begins with determining the optimal 9 or 12 parameter affine transformation to register the images. This step is done in SPM by minimising the sum of squared differences between the images χ^2 . Normalisation isn't quite straightforward, without constraints and with poor data, a simple parameter approach can produce some extremely unlikely transformations. For example when there are only a few transverse slices in the Z direction it is extremely difficult to determine an accurate zoom in this direction. It is possible to simply assign a fixed value for this difficult-to-determine parameter and fit for the remaining ones.

Another possibility is to incorporate prior information into the optimisation procedure whereby a smooth transition between fixed and fitted parameters can be achieved. When the error for a particular fitted parameter is known to be large, then the parameter will be based more upon the prior information. This approach is known as the Bayesian approach [4].

4 Statistical analysis

In this final stage the **neurophysiological** responses are partitioned into components of interest, confounds or components of no interest, and an error term. The partitioning involves estimating the components in terms of parameters associated with the design matrix (see below). The analysis of regionally specific effects uses the general linear model to assess the contribution or differences among parameter estimates (specified by a contrast), by referring to the error variance. This assessment is in terms of an F or t value for voxel (i.e. a SPM{F} or SPM{t}). The SPM{t} is transformed to the unit normal distribution to give a Gaussian field SPM{Z}.

There are some standard procedures, using the theory of Gaussian fields, which can be used on SPM{Z} for statistical analysis. These procedures give p-values (probability values) that refer to different levels of inference; these levels of inference can be in terms of (i) the number of activated regions (number of clusters above some peak and spatial threshold), (ii) the number of activated voxels in a particular region and (iii) the p-value for each voxel within that region. The p-values are corrected for the multiple non-independent comparisons in the analysis, arising from the fact that there is a dependence between the different voxels. The final result of the analysis consists of the SPM{Z} and the associated probability values.

4.1 The general linear model

The general linear model [13] is an equation that relates what one observes, to what one expects to see, by expressing the observations (*response variable*) as a linear combination of expected components (*explanatory variables*) and some residual error. The general linear model for a response variable x_{ij} such as rCBF at voxel $j = 1, \dots, J$ is:

$$x_{ij} = g_{i1}\beta_{1j} + g_{i2}\beta_{2j} + \dots g_{iK}\beta_{Kj} + e_{ij} \quad (1)$$

where $i = 1, \dots, I$ indexes the observations (e.g. scans). The general linear model assumes the errors (e_{ij}) are independent and identically distributed variables with distribution $[N(0, \sigma_j^2)]$. In activation studies this means assuming an equal error variance (σ_j^2) across conditions and subjects (but not from one voxel or brain structure to the next). β_{kj} are K unknown parameters for each voxel j that represent the relative contribution of each of the explanatory variables.

The coefficients g_{ik} are explanatory variables relating to the conditions under which the observation (scan) i was made. The coefficients can be of two sorts: (i) a *covariate* (e.g. global CBF, time, plasma prolactin level, ... etc) in which case equation(1) is a familiar multivariate regression model or (ii) indicator-type

or dummy variables, taking integer values to indicate the level of a factor (e.g. condition, subject, drug ... etc) under which the response variable (e.g. perfusion) is measured. Equation(1) can be written in matrix form as a multivariate general linear model:

$$X = G\beta + e$$

The least square estimate of β , denoted by b , satisfies the equation:

$$G^T G b = G^T X$$

if G is of full rank then $G^T G$ is invertible and the least squares estimate is given uniquely by

$$b = (G^T G)^{-1} G^T X \quad (2)$$

The mean and variance of b are given by $E\{b_j\} = \beta_j$ and $Var\{b_j\} = \sigma_j^2 (G^T G)^{-1}$

If the errors are normally distributed then the least square estimates are also the maximum likelihood estimates and are themselves normally distributed [8]. $E\{b_j\}$ is simply the expectation or average of the parameter estimates. $Var\{b_j\}$ is the variance-covariance matrix for the parameter estimates corresponding to the j th voxel. These equations can be used to implement a vast range of statistical analyses. The issue is therefore not so much the mathematics but the formulation of a design matrix G appropriate to the study design and inferences that are sought.

The design matrix can contain both covariates and indicator variables reflecting the experimental design. Each column of G has an associated unknown parameter in the vectors β_j . Some of these parameters will be of interest [e.g. the effect of a particular sensorimotor or cognitive condition or the regression coefficient of rCBF (the response variable) on reaction time (covariate)]. The remaining parameters will be of no interest and pertain to confounding effects (e.g. the effect of being a particular subject or the regression slope of voxel activity on global activity). Confounding here is used for an uninteresting effect that could confound the estimation of interesting effects (e.g. a nuisance effect like global activity).

4.2 Statistical inference

The two levels (indicator vs. covariate and interesting vs. not interesting) suggest that G and β can be split into: $G = [G_I G_c H_I H_c]$ and $\beta = [\beta_I \beta_c \gamma_I \gamma_c]^T$, where

the subscripts l and c refer to the nature of the effects (level within a factor or a covariate). G_α denotes the effects of interest and H_α denotes the confounding effects of no interest ($\alpha = l, c$). Similarly β_α are the effects of interest and γ_α are the effects of no interest.

Using the partitions as described we can write the response matrix:

$$X = G_l\beta_l + G_c\beta_c + H_l\gamma_l + H_c\gamma_c + e$$

G_l just represents a matrix of zeros and ones. This particular matrix indicates the presence of rest or the presence of activation. $G = G_l$ can be used for a simple test. If we want to test for example a regression of reaction time on rCBF, $G = G_c$ can be used where G_c is a column vector containing the reaction time data.

The randomized block design ANCOVA implemented by the SPM software corresponds to $G = [G_l H_l H_c]$, where G_l specifies the rest/activation condition, H_l accounts for subject (block) effects and H_c is a column vector of confounding global CBF covariates.

After calculating β using X we can calculate an adjusted X' where we discount the effects of no interest:

$$X' = X - [H_l H_c][g_l^T g_c^T]^T$$

$H_l H_c$ are the effects of no interest with corresponding parameter estimates $[g_l^T g_c^T]^T$ where g_l and g_c are the linear estimates of γ_l and γ_c .

The hypothesis that the effects given by G_l and G_c are not significant can be tested in the following way. The sums of squares and products due to error is obtained from the difference between the actual and the estimated value of X :

$$S = (X - Gb)^T(X - Gb)$$

The error sum of squares and products under the null hypothesis are given by

$$S_0 = (X')^T(X')$$

which expands to

$$S_0 = (X - [H_l H_c][g_l^T g_c^T]^T)^T(X - [H_l H_c][g_l^T g_c^T]^T)$$

From this point we can test the omnibus significance of all effects of interest over all voxels. This corresponds to a multivariate analysis of variance or covariance (MANOVA or MANCOVA). A second approach, which is used by SPM is to test for omnibus significance over effects of interest in a univariate sense at each voxel (ANOVA or ANCOVA). This second test is achieved by statistical parametric mapping and preserves the regional specificity of the omnibus test. To test for

the omnibus significance over effects of interest in a univariate sense at each voxel we use the F statistic (which is the univariate ratio of variance [5])

$$F_j = \frac{(S_{0(j)} - S_j)/(r_0 - r)}{S_j/r}$$

where $S_{0(j)} - S_j$ is the measure of the variation between the means of respectively the rest- and the activation group. S_j is the measure of the data within the activation group.

F_j is distributed according to the F distribution with degrees of freedom $r_0 - r$ and r . Here $r = I - \text{rank}(G)$ and $r_0 = I - \text{rank}([H_l H_c])$, where I is the total number of scans.

The resulting F_j is displayed as an image, the $\text{SPM}\{F\}$. This $\text{SPM}\{F\}$ tests the overall significance of all effects which are of interest. In The SPM program the $\text{SPM}\{F\}$ is only used for selecting subsets of voxels that are used in some further analysis.

4.2.1 Contrasts

For further analysis we test the significance of specific effects. Suppose that the parameter estimates at voxel j are $b_j = [b_{1j}, b_{2j}, \dots]^T$. Now we can test for specific effects using linear compounds or *contrasts* of these parameter estimates. For example if we want to test for activation of condition 2 in comparison to condition 1 we would use contrast $c = [-1 \ 1 \ 0 \ 0 \dots]$. We can use any kind of contrast, but the contrast vector should sum to zero ($\sum c_i = 0$). The significance of a particular linear compound of effects at voxel j is tested with:

$$t_j = \frac{c \cdot b_j}{\varepsilon_j}$$

For G of full rank $c^T b \sim N(c^T \beta, \sigma^2 c^T (G^T G)^{-1} c)$ The residual variance σ^2 is estimated by the residual mean square, the residual sum-of-squares divided by the appropriate degrees of freedom: $\hat{\sigma} = \frac{S_j}{r}$. This gives the following approximation of the standard error:

$$\varepsilon_j^2 = (S_j/r) c (G^T G)^{-1} c^T$$

t_j has the Student's t distribution with degrees of freedom r . Thus the null hypothesis: $\mu_1 = \mu_2$ is equivalent to $S_0: c^T \beta = 0$ with $c = (-1, 1)^T$. This gives a T-statistic:

$$T_j = \frac{\mu_{2j} - \mu_{1j}}{\sqrt{\varepsilon_j^2}}$$

By equation(2) this can be written as:

$$T_j = \frac{\beta_{2j} - \beta_{1j}}{\sqrt{\varepsilon_j^2}}$$

T_j can be displayed as an image, the $\text{SPM}\{t\}$ and represents a spatially extended statistical process that directly reflects the significance of the effects described by contrast c .

Finally the $\text{SPM}\{t\}$ can be transformed to a Gaussian distribution. If the degrees of freedom of the original distribution are reasonably high the resulting SPM approximates a Gaussian field or $\text{SPM}\{Z\}$. This transformation can be done using a probability integral transform [26].

4.3 Spatial extent as additional tool to test significance of activation

The most common method for interpretation of an SPM is thresholding. A threshold is used such that for an SPM of a given size, the probability of obtaining one or more activation areas of at least one voxel, by chance, is suitable small (for example 0.05). The problem is that activation areas are not only characterized by threshold, but also by their spatial topography. For this reason the detectability of significant areas would be enhanced if the volume of activated tissue was explicitly included when testing the null hypothesis that the activated region could have occurred by chance.

More precisely, for any region, one would like to ask: ‘Over a SPM of a given size, what is the probability under the null hypothesis of obtaining one or more activation areas of the same size, or larger, than the one in question?’ It is also interesting to know what the relation is between the height threshold and the volume threshold. The solutions to these questions will be given by the theory which follows:

The particular probability we are interested in is: *The probability of obtaining at least one activation with k voxels or more.* This can be written as the probability that the largest region has k voxels or more $= P(n_{\max} \geq k)$, where n_{\max} is the number of voxels in the biggest region. This probability is a more general case of the probability of getting at least one activation (with one voxel or more) $= P(n_{\max} \geq 1)$. This is equal to the probability of getting at least one region $P(m \geq 1)$, where m is the number of activated areas. $P(m \geq 1)$ is usually set to 0.05 by choosing an appropriate threshold or critical height u . This threshold is chosen by estimating the expectation or mean of m and using the fact that

$$P(m \geq 1) \leq E\{m\}$$

tends to equality at high thresholds [15]. Suppose n is the number of voxels in each cluster. The problem addressed is how to estimate $P(n_{\max} \geq k)$ and obtain a critical threshold for volume. To calculate $P(n_{\max} \geq k)$ we simply compute one minus the probability that all the m regions have less than k voxels, times the probability of getting m regions. These probabilities are summed over all

possible values of m :

$$P(n_{max} \geq k) = \sum_{i=1}^{\infty} P(m = i) \cdot [1 - P(n < k)^i] = 1 - e^{-E\{m\} \cdot P(n \geq k)} \quad (3)$$

$P(n \geq k)$ can be calculated using $P(n = x)$, $P(n = k)$ can be assumed by finding a form which is asymptotically correct and whereby the parameters are determined by reference to its known moments:

$$P(n = x) \approx \frac{2\beta}{D} x^{2/D-1} e^{-\beta x^{2/D}}$$

where D is the dimension of the SPM. We now can write:

$$P(n \geq x) \approx e^{-\beta x^{2/D}}$$

By a change of variables β can be calculated using $E\{n\}$ (see [14]) and the fact that

$$E\{N\} = E\{m\} \cdot E\{n\}$$

where N is the number of voxels above the threshold u , m is the number of activated regions and n is the number of voxels in each cluster. $E\{m\}$ and $\{N\}$ are known (see [14]) which gives a formula for β . Now we have a solution for $P(n_{max} \geq k)$:

$$P(n_{max} \geq k) = 1 - e^{-E\{m\} \cdot e^{-\beta k^{2/D}}}$$

We now have an equation that gives an estimate of the probability of finding at least one region with k or more voxels in an SPM. The next thing to do is to determine the threshold u . An optimum threshold should maximize sensitivity. The article [14] derives an approximate expression for the sensitivity to a 'random' signal. The article shows that broader signals are best detected by low thresholds and that sharp focal signals are best detected by high thresholds. Plots are given also to show how sensitivity depends on the height threshold and the region-size. The results show that using a lower height threshold more activation areas could be detected when the activation topography is taken into account. This approach frees the analysis of significant focal change from the arbitrary nature of high thresholds which gives a substantial increase in sensitivity.

SPM generates different kinds of probability values. The p-values are given dependent on both cluster size and height threshold, the p-values which only depend on the height threshold are also given. Both these p-values are given corrected for the fact that there is a dependence between the different voxels and uncorrected for this dependence.

5 Analysing the data

5.1 The fMRI data

The data comes from an experiment with a subject performing some memory tasks, where the subject had to remember visual patterns. While time passed, the subject was alternating between a rest condition and an activation condition, with a total of thirteen periods. Each condition lasted 42 seconds and a total of six volume scans were made in each condition. The experiment was set up in the following way:

- 6 images: subject in rest (only a fixation point)
- 6 images: subject performing a memory task (brightness patterns)
- 6 images: subject in rest
- 6 images: subject performing an alternative memory task (character)
- 6 images: subject in rest (only a fixation point)
- 6 images: subject performing a memory task (red/green patterns)
- 6 images: subject in rest
- 6 images: subject performing an alternative memory task (character)
- 6 images: subject in rest (only a fixation point)
- 6 images: subject performing a memory task (blue/yellow patterns)
- 6 images: subject in rest
- 6 images: subject performing an alternative memory task (character)
- 6 images: subject in rest

The volume scans consist of 24 slices with a resolution of 128×128 pixels and a voxel-size of (2, 2, 4) mm. The Z-area covered almost the entire brain. The scans were not taken parallel to the x-y plane. There was a little rotation around the x-axis, which can be seen in the output of the normalisation.

A total of 4 datasets are available where this experiment is performed. Also, the 4 datasets are merged into an overall dataset to improve the accuracy of the statistical tests.

5.2 Preprocessing the data

The preprocessing steps: registration, normalisation and smoothing have to be performed for each dataset. After preprocessing, the datasets are combined by SPM by treating each dataset as a separate subject. Before preprocessing can be performed in the SPM program the *raw* data has to be moved to the **analyze** format which can be read by SPM.

5.2.1 Moving the *raw* data to analyze files

The *raw* data files have the following format:

Roland_BP_sxxx_iyyyy.ima

xxx runs from 005 to 082 for the first dataset, from 083 to 160 for the second dataset and so on.

yyyy runs from 0077 to 1948 for the first dataset, from 1949 to 3820 for the second dataset and so on.

To make the *raw* data more manageable the files are transformed to the format:

rolxx-yy.ima

xx numbers the 78 volumes, beginning by 01. yy numbers the 24 scans, beginning by 01. For each dataset these files are the same and therefore are placed in separate *directories*.

The next step is to transform the *raw* data files to the **analyze** format, this is done with the program **medcon** [23]:

```
medcon -c "anlz" -f rol0*.ima
medcon -c "anlz" -f rol1*.ima
medcon -c "anlz" -f rol2*.ima
medcon -c "anlz" -f rol3*.ima
medcon -c "anlz" -f rol4*.ima
medcon -c "anlz" -f rol5*.ima
medcon -c "anlz" -f rol6*.ima
medcon -c "anlz" -f rol7*.ima
```

The last step is to make a header for the **analyze** files and to put the scans into a volume. To make a header a program of **AIR** [28] is used:

```
makeaheader rolxx.hdr 2 128 128 24 2 2 4
```

xx denotes the volume number, the next parameter 2, selects 16bits/pixel, next to follow are the size of the scan, the number of scans and the voxel size. Finally we combine the scans with the **UNIX** **cat** command:

```
cat *rolxx*.img >rolxx.img
```

5.2.2 Realignment

The first step is to realign the data. The realignment in SPM is done in 2 steps.

1. **coregister** The parameters are determined to realign the selected images to the first image in the selected series. The transformation parameters for each image *filename.img* are saved in *filename.mat*. Current *.mat* files should first be deleted, otherwise the *.mat* files are used for the starting point of the transformation and updated.
2. **reslice** This step involves rotating and translating the images so that they coincide with the first image in the selected series. The transformation has as input *filename.img*, it uses *filename.mat* for the transformation parameters and the file is saved as *rfilename.img*

These two steps can be carried out separately or combined. Because the computer used to process the data didn't have enough memory, the steps had to be carried out separately.

There were 4 datasets to be realigned. The scans in all the 4 datasets were realigned to the first scan in the first dataset, because the scans between different datasets have also to be in register for a proper statistical analysis.

Due to the memory problem the reslicing had to be further divided in small steps. The reslicing of the 78 scans was done in 3 steps per dataset.

5.2.3 Coregister

SPM has the option to realign between images of different modality as well. This is especially useful if the normalisation is not based on the functional data, but on another kind of scan (for example a structural scan). Coregistration in SPM is done in the same 2 steps as for realignment.

1. **coregister** The parameters are determined to realign the selected images to the first image in the selected series. The transformation parameters for each image *filename.img* are saved in *filename.mat*. Current *.mat* files should first be deleted, otherwise the *.mat* files are used for the starting point of the transformation and updated.
2. **reslice** This step involves rotating and translating the images so that they coincide with the first image in the selected series. The transformation has as input *filename.img*, it uses *filename.mat* for the transformation parameters and the file is saved as *rfilename.img*

For coregistration some extra parameters have to be filled in. The modality of the target- and the modality of the object image is requested. These modalities can be PET, T1 MRI or T2 MRI.

In our case coregistration was not necessary, because the normalisation was based

on a functional scan. Normally when normalisation is done on a scan of different modality and coregistration is not used, then there can be a small difference between the place of the real activity and the place of the activity shown in the 'glass' brain².

5.2.4 Normalize

Normalisation in SPM is done in 2 steps:

1. **Determine parameters** The parameters are determined to normalize an image to a template. The parameters for the normalisation are saved in *filename_sn3d.mat*.
2. **Write normalised Reslices** and saves images using the normalisation parameters specified by the selected *_sn3d.mat* file. The normalisation parameters are applied to the selected images *filename.img* and saved in the files *nfilename.img*

We normalised the mean scan of some of the functional scans to a EPI-template, because a visual inspection showed that the EPI-template matched the functional scans best.

A mean scan of all the functional scans was not available, because the realignment was carried out in smaller steps.

Standard normalisation was not used. The nonlinear normalisation was skipped totally, because of an unsatisfying result when observing the output of the normalisation. The normalisation was done with an 9 parameter (6 rigid body parameters + x-, y- and y-zooms) transformation.

A sinc interpolation is used to resample the images to get a more accurate result.

5.2.5 Changed defaults for normalisation

- Custom starting estimates

Starting estimates are: [xt yt zt xr yr zr xs ys zs xa ya za]
(t=transformation, r=rotation, s=scaling, a=affine, in x, y and z). The default starting estimates are [0 0 0 0 0 0 -1 1 1 0 0 0]. To normalize the data these parameters are manually changed to [0 0 0 0 0 0 -1 -1 -1 0 0 0]. The z-scaling is negated to flip the data in the z-direction, because the slices were upside-down. The y-scaling is negated to flip the back and the front of the brains. It isn't known if the x-direction should be flipped, this can not be seen with the naked eye. Background information should be provided to know for certain, whether the x-direction flip is justified.

²x-, y- and z-view of a standard brain where just the surroundings of the brain are shown as a reference to show the activity in the brain

- Voxel-size

The voxel-size is denoted by (x size, y size, z size). For the data the voxel-size has been manually changed to (2, 2, 2) (mm). The original data had a voxel-size of (2, 2, 4) (mm). The normalisation has been adjusted to resample the data to the new voxel-size, to get more slices in the z-direction. The reason for this is that the slices will be resampled with a higher accuracy after normalisation, due to the fact that the slices had a little rotation in the x-direction before normalisation.

The voxel-size could be set lower, but it would take a lot more time to normalize the data. Taking this into account and the fact that the data will be smoothed before doing the statistical analysis, a voxel-size of (2, 2, 2) (mm) was chosen.

5.2.6 Smoothing

The normalised images are smoothed to increase signal to noise ratio. The suggested value for the Gaussian filter width (FWHM) is 2 to 3 times the voxel-size. For our data a FWHM of 3 times the voxel-size was used in the x-, y- and z-direction.

5.3 The tests

A total of 5 tests were performed with the data. The layout for the first test of the experiment is as follows:

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| R | M | R | M | R | M | R | M | R | M | R | M | R |
|---|---|---|---|---|---|---|---|---|---|---|---|---|

where: **R** denotes the rest scans, **M** denotes the scans where the subject performs a memory task (remembering brightness- and colour patterns) or an alternative memory task (remembering visual presented characters).

For these tests we use 1 2 1 2 1 2 1 2 1 2 1 2 1 as the order of epochs for all of the 4 datasets. The following test was carried out:

1. Testing for activation due to the memory task against the rest condition.
This was done using contrast -1 1 (see section 4.2.1) in all of the 4 datasets.

The layout for the other 4 tests of the experiment is as follows:

| | | | | | | | | | | | | |
|---|---|---|----|---|---|---|----|---|---|---|----|---|
| R | M | R | AM | R | M | R | AM | R | M | R | AM | R |
|---|---|---|----|---|---|---|----|---|---|---|----|---|

where: **R** denotes the rest scans, **M** denotes the scans where the subject performs a memory task (remembering brightness- and colour patterns) and **AM** denotes the scans where the subject performs an alternative memory task (remembering

visual presented characters).

For these tests we use 1 2 1 3 1 2 1 3 1 2 1 3 1 as the order of epochs for all of the 4 datasets. The following tests were carried out:

- 2 Testing for activation due to the memory task against the rest condition. This was done using contrast -1 1 0 in all of the 4 datasets.
- 3 Testing for activation due to the alternative memory task against the rest condition. This was done using contrast -1 0 1 in all of the 4 datasets.
- 4 Testing for activation due to the memory task, without the activated areas caused by the alternative memory task. This was done using contrast -1 2 -1 in all of the 4 datasets.
- 5 Testing for activation due to the alternative memory task, without the activated areas caused by the normal memory task. This was done using contrast -1 -1 2 in all of the 4 datasets.

5.4 fMRI statistics options

The following parameters have to be filled in. The correct values for the parameters depend on the data and the kind of tests which have to be performed.

- Interscan interval

This is the time between consecutive volume acquisitions. In other words, this is the time needed to scan all the slices in a volume. For our data the interscan interval equals 7 seconds.

- Number of subjects

The data we use comes from a single person, but because we have 4 datasets we select 4 as the number of subjects.

- Type of response

We can choose from the following responses:

- 2 temporal basis functions
- Half sine wave
- delayed box-car

For our data we select the delayed box-car, because this response just alternates between two conditions. The delayed box-car response is a fixed

response function characterised by a square waveform. The waveform represents a transient response to epoch onset and lasts for the duration of that epoch. Each covariate expresses the same waveform every time the condition occurs. The delayed box-car response can be seen at the detailed results in section 5.6.1.

- Order of epochs

The successive conditions or epochs have to be entered in presentation order. The epochs are not numbered in the same way, during the different tests. In the first test we don't make a distinction between the different memory tasks, but in the 4 tests which follow we do (see section 5.3).

- Scans per epoch

The scans per epoch have to be entered in presentation order. For our data all the epochs consist of 6 scans. So we just select 6.

- Covariates of no interest

The covariates of no interest (or confounds) can be entered, for our data we don't select any covariates of no interest.

- High pass filter

We use a high pass filter to remove low frequency artefacts in time. The cut-off period in seconds for the high pass filter should be approximately 2 times the length of the rest-activation cycle (see [16], page 13). This cycle is 84 seconds for our data, so we select a cut-off period of 168 seconds.

- Global normalisation

To correct for global brain activity we use global normalisation. There is only a single session, so we select AnCova to do the normalisation, as suggested by the authors of SPM.

- Contrasts

The number of contrasts has to be entered and for each contrast a vector list of coefficients has to be entered. For each test a different contrast is used, these contrasts are shown in section 5.3.

- Temporal Smoothing

Temporal smoothing uses a kernel that approximates the **hemodynamic** response to maximise signal variance components relative to noise. This option should be used when the scan interval is less than 6 seconds. Our data is scanned with a scan interval of 7 seconds, so we don't use this option.

5.5 SPM output

On the next 2 pages follows the output produced by SPM when doing the realignment and the normalisation of the data. The output is presented in the following order:

Realignment This output describes the transformation used to realign the scans.

Normalisation A visual result of the normalisation is given and the calculated parameters used to do the normalisation are shown.

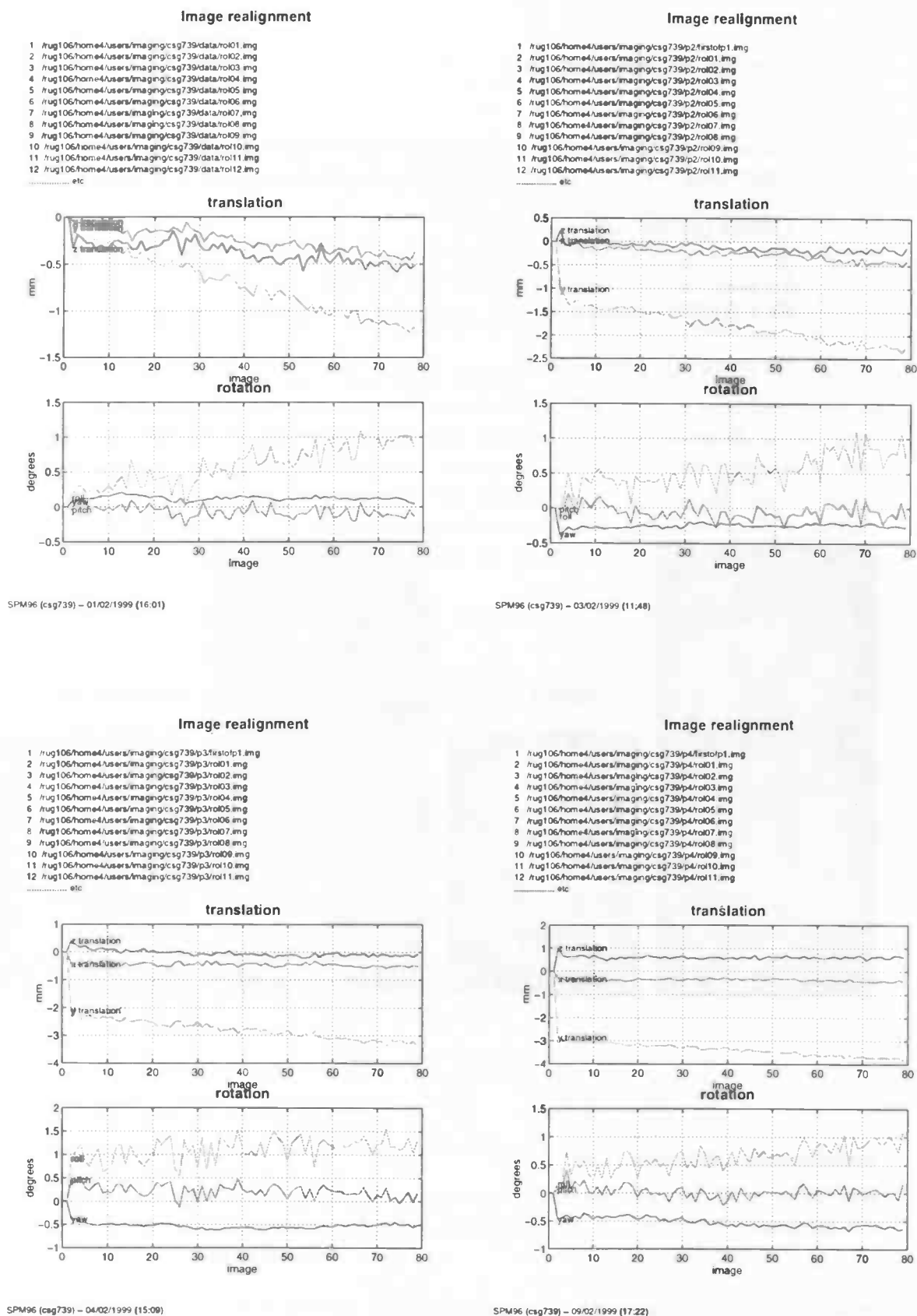


Figure 1: The realignment of the 4 datasets with reference to the first scan in the first dataset. The first dataset can be seen top-left, the 4th dataset bottom-right. It can be seen that the subject moves slightly, especially in the y-direction.

Spatial Normalisation

Image : /rug106/home4/users/imaging/csg739/data/meanrol01.img

Parameters : /rug106/home4/users/imaging/csg739/data/meanrol01_sn3d.mat

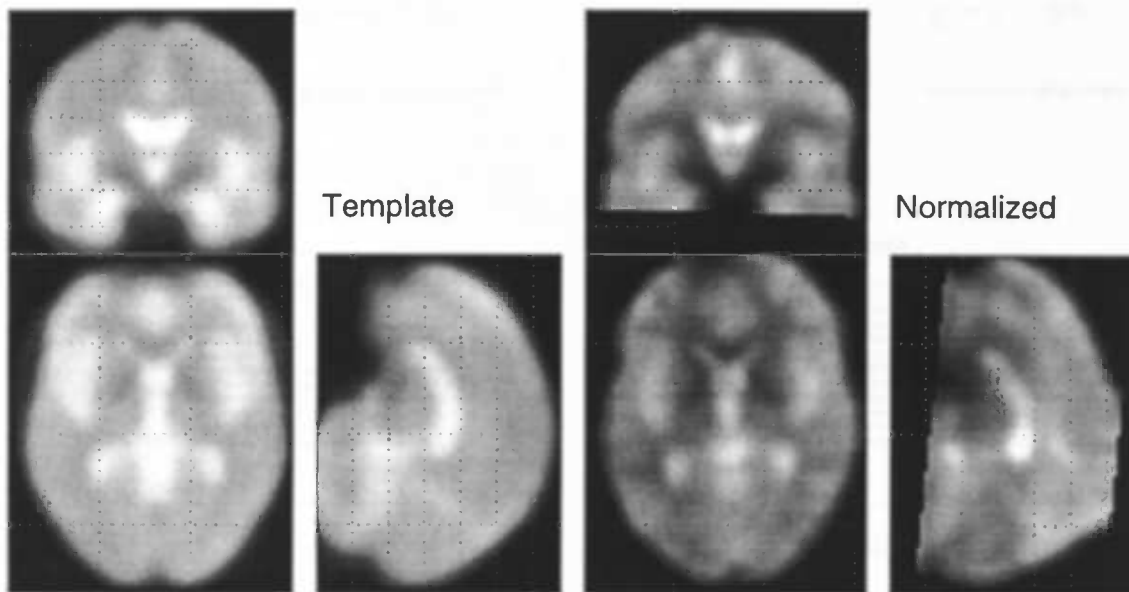
Linear {affine} component

$$X1 = -1.10 \cdot X + 0.06 \cdot Y + -0.03 \cdot Z + -0.27$$

$$Y1 = -0.06 \cdot X + -0.99 \cdot Y + 0.11 \cdot Z + -21.05$$

$$Z1 = 0.03 \cdot X + -0.10 \cdot Y + -1.11 \cdot Z + 24.30$$

No nonlinear components



SPM96 (csg739) - 02/02/1999 (14:44)

Figure 2: The normalisation of the 4 datasets. For each dataset we see the template on the left and the normalised data is shown on the right side. Both the template and the normalised data are shown in 3-views (top left: the frontal view, bottom left: the view from above and bottom right: the view from the side)

5.6 Results

The SPM outputs of the tests are available on the next pages. SPM prints SPM{F} maps as well, but they are skipped here, because SPM only uses the SPM{F} maps to select voxels for further analysis.

For each test the following information is shown:

Contrast The used contrast is displayed on top of the design matrix.

Design matrix The design matrix is shown. The first row of the matrix describes the activation- and rest conditions. The second row and some of the rows which follow describe the activation in more detail, if the activation is subdivided in different kinds of activation. For example in test 2,3,4 and 5 we see 2 rows with the different activations for memory and alternative memory. The highpass filter is shown in the rows which follow. The final row shows the data used by Ancova to correct for global brain activity.

For our data we see a big design matrix where the design matrices of the different datasets are combined.

Statistics The results show the activation in SPM{Z}. For each cluster a detailed description of P values & statistics is shown, which will be explained in an example below.

When displaying the results height- and extent thresholds must be given. These can be given as p-values or respectively as Z-values or voxel numbers.

For the tests standard p-values were used. This is a p-value of 0.01 and an extent threshold which depends on the number of expected voxels per cluster. For our data only the clusters are shown of which the number of voxels exceeds 26.

For all the tests the P values & statistics are given. These values will be explained below for the first cluster in test 1:

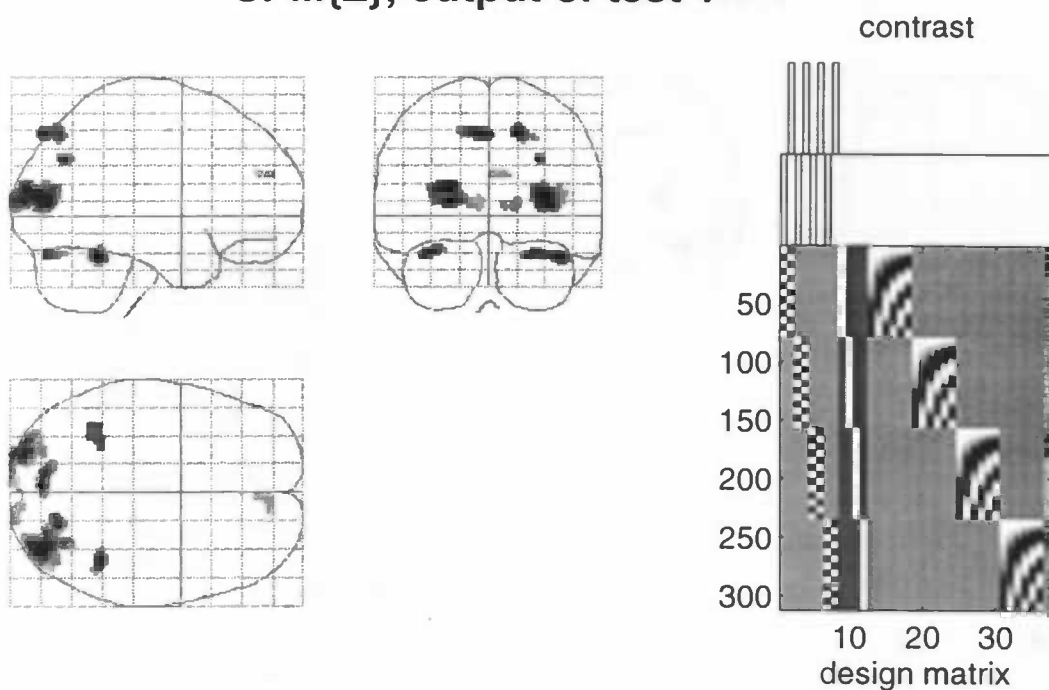
| cluster-level{k,Z} | voxel-level{Z} | uncorrected k & Z | x,y,x {mm} |
|--------------------|----------------|-------------------|------------|
| 0.001 (370, 7.05) | 0.000 (7.05) | 0.001 0.000 | 34 -78 8 |

These values should be interpreted in the following way:

- The probability of getting, by chance, a cluster of size 370 or more and a maximum Z-value of 7.05 or more in this cluster is 0.001 (corrected for multiple comparisons)
- The probability of getting, by chance, a maximum value of 7.05 in the entire image is 0.000 (corrected for multiple comparisons)

- The probability of getting, by chance, a cluster of size 370 or more is 0.001. Conditional on there being a suprathreshold cluster in the first place (thus not corrected for multiple comparisons).
- The probability of getting, by chance, a Z value of 7.05 or more is 0.000 (not corrected for multiple comparisons). This value is obtained by looking the Z-value up in statistical tables for the standard normal distribution.

SPM{Z}, output of test 1



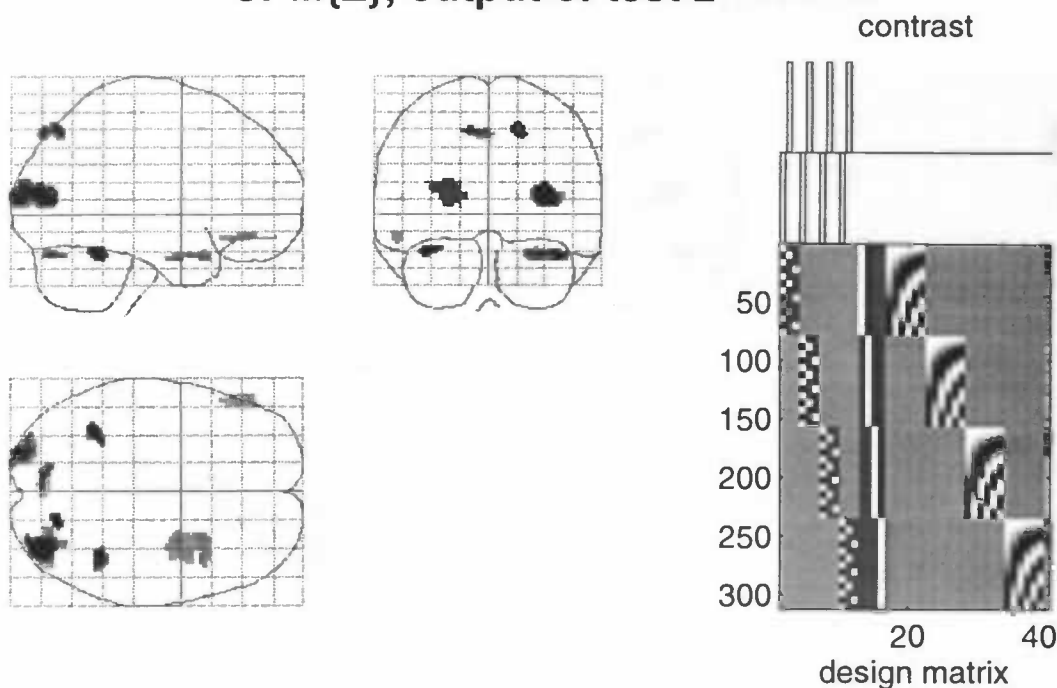
P values & statistics: ../rug106/home4/users/imaging/csg739

| set-level {c} | cluster-level {k,Z} | voxel-level {Z} | uncorrected k & Z | | x,y,z {mm} | | |
|---------------|---------------------|------------------|-------------------|-------|------------|------|-----|
| 0.998 (10) | 0.001 (370, 7.05) | 0.000 (7.05) | 0.001 | 0.000 | 34 | -78 | 8 |
| | | 0.000 (6.89) | | 0.000 | 34 | -86 | 14 |
| | | 0.417 (4.18) | | 0.000 | 42 | -72 | 18 |
| | 0.004 (320, 6.43) | 0.000 (6.43) | 0.001 | 0.000 | -22 | -92 | 18 |
| | | 0.000 (6.10) | | 0.000 | -24 | -94 | 10 |
| | | 0.036 (4.84) | | 0.000 | -16 | -100 | 12 |
| | | 0.008 (51, 6.07) | 0.146 | 0.000 | 42 | -48 | -26 |
| | 0.011 (90, 5.99) | 0.000 (5.99) | 0.060 | 0.000 | -4 | -80 | 48 |
| | 0.012 (62, 5.90) | 0.000 (5.90) | 0.112 | 0.000 | 18 | -72 | 50 |
| | | 0.941 (3.70) | | 0.000 | 28 | -72 | 46 |
| | 0.013 (59, 5.85) | 0.000 (5.85) | 0.120 | 0.000 | 28 | -78 | -22 |
| | 0.040 (55, 5.38) | 0.003 (5.38) | 0.132 | 0.000 | -36 | -52 | -22 |
| | 0.126 (27, 5.06) | 0.014 (5.06) | 0.284 | 0.000 | 30 | -68 | 34 |
| | 0.285 (27, 4.53) | 0.133 (4.53) | 0.284 | 0.000 | 16 | -98 | 8 |
| | 1.000 (31, 3.11) | 1.000 (3.11) | 0.251 | 0.001 | 4 | 52 | 24 |

Height threshold {u} = 2.33, $p = 0.010$
 Extent threshold {k} = 25 voxels, $p = 0.302$
 Expected voxels per cluster, $E\{n\} = 25.4$
 Expected number of clusters, $E\{m\} = 21.3$

Volume {S} = 178896 voxels or 1665.4 Resels
 Degrees of freedom due to error = 279.0
 Smoothness FWHM {mm} = 12.1 11.9 6.0
 {voxels} = 6.0 5.9 3.0

SPM{Z}, output of test 2

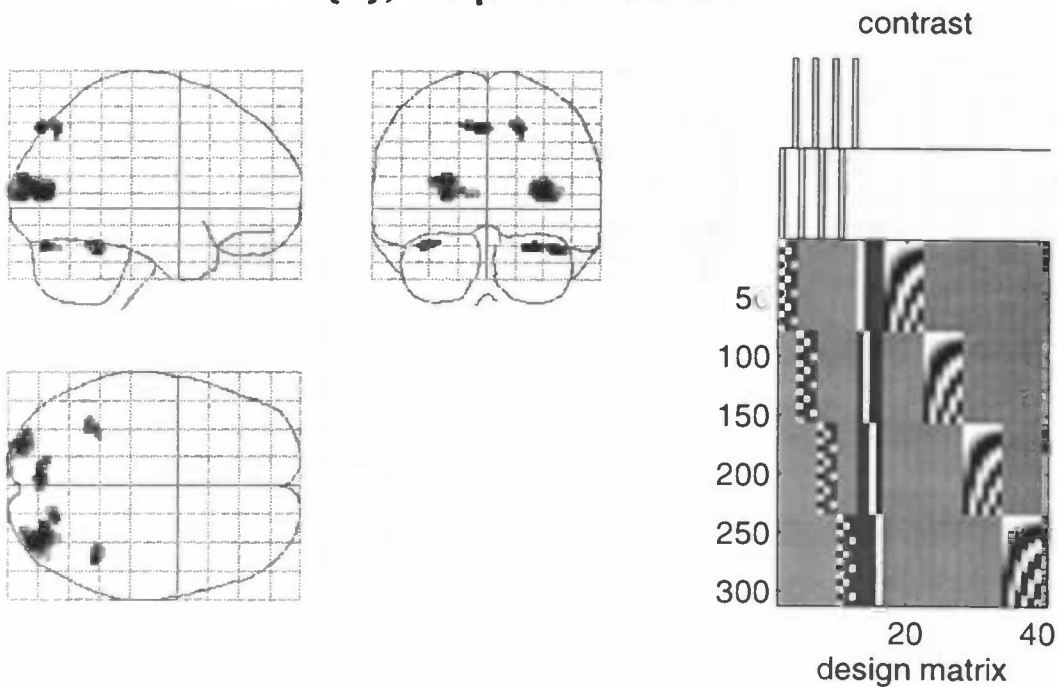


P values & statistics: ./rug106/home4/users/imaging/csg739

| set-level {c} | cluster-level {k,Z} | voxel-level {Z} | uncorrected k & Z | | x,y,z {mm} | | |
|---------------|---------------------|-----------------|-------------------|-------|------------|-----|-----|
| 0.999 (9) | 0.022 (251, 5.65) | 0.001 (5.65) | 0.004 | 0.000 | 34 | -78 | 8 |
| | | 0.001 (5.62) | | 0.000 | 36 | -86 | 14 |
| | 0.072 (35, 5.15) | 0.009 (5.15) | 0.227 | 0.000 | -38 | -52 | -22 |
| | 0.067 (185, 5.14) | 0.009 (5.14) | 0.011 | 0.000 | -24 | -96 | 10 |
| | | 0.031 (4.87) | | 0.000 | -24 | -90 | 16 |
| | | 0.795 (3.87) | | 0.000 | -32 | -90 | 18 |
| | 0.070 (43, 5.12) | 0.010 (5.12) | 0.182 | 0.000 | 40 | -48 | -24 |
| | 0.093 (33, 5.04) | 0.015 (5.04) | 0.240 | 0.000 | 18 | -72 | 52 |
| | 0.464 (39, 4.30) | 0.294 (4.30) | 0.203 | 0.000 | 32 | -76 | -24 |
| | 0.689 (50, 4.08) | 0.543 (4.08) | 0.152 | 0.000 | -10 | -80 | 50 |
| | | 0.953 (3.67) | | 0.000 | -2 | -82 | 48 |
| | 0.969 (110, 3.22) | 1.000 (3.22) | 0.042 | 0.001 | 30 | 0 | -26 |
| | | 1.000 (3.08) | | 0.001 | 36 | 14 | -26 |
| | | 1.000 (2.83) | | 0.002 | 38 | 4 | -24 |
| | 1.000 (28, 2.99) | 1.000 (2.99) | 0.279 | 0.001 | -56 | 40 | -14 |

Height threshold {u} = 2.33, p = 0.010
 Extent threshold {k} = 26 voxels, p = 0.296
 Expected voxels per cluster, E(n) = 25.8
 Expected number of clusters, E(m) = 20.6

Volume {S} = 178896 voxels or 1642.2 Resels
 Degrees of freedom due to error = 275.0
 Smoothness FWHM (mm) = 12.1 11.9 6.0
 {voxels} = 6.0 6.0 3.0



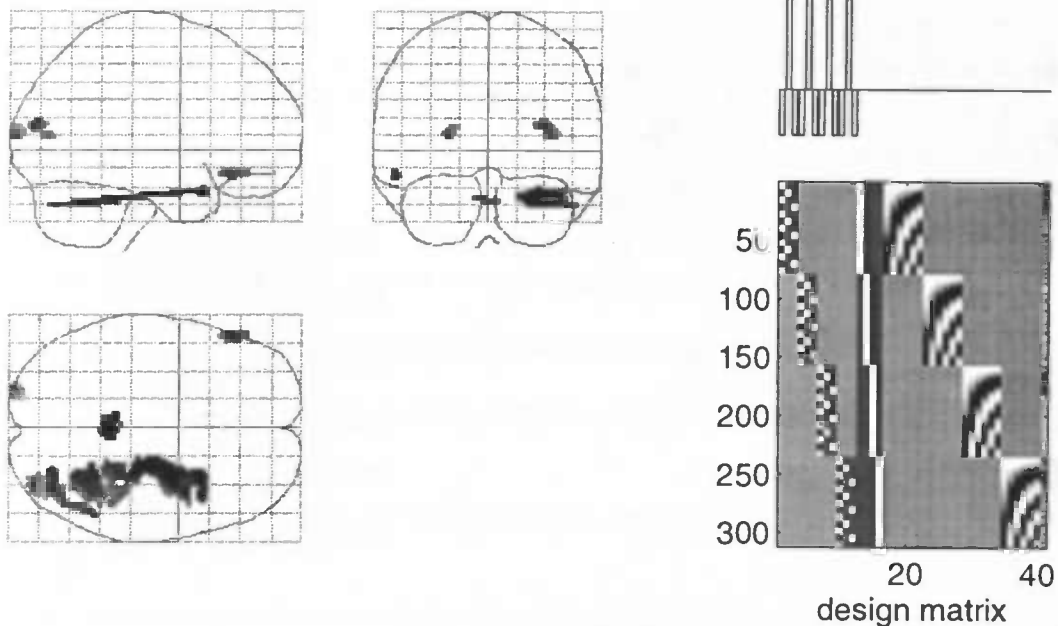
P values & statistics: ../rug106/home4/users/imaging/csg739

| set-level {c} | cluster-level {k,Z} | voxel-level {Z} | uncorrected k & Z | | x,y,z {mm} | | |
|---------------|---------------------|-----------------|-------------------|-------|------------|------|-----|
| 1.000 (7) | 0.009 (248, 6.04) | 0.000 (6.04) | 0.004 | 0.000 | 32 | -80 | 10 |
| | 0.018 (52, 5.71) | 0.001 (5.71) | 0.145 | 0.000 | -4 | -80 | 48 |
| | 0.034 (38, 5.54) | 0.001 (5.54) | 0.209 | 0.000 | 28 | -78 | -22 |
| | 0.056 (194, 5.22) | 0.006 (5.22) | 0.010 | 0.000 | -22 | -92 | 18 |
| | | 0.024 (4.93) | | 0.000 | -24 | -92 | 10 |
| | | 0.822 (3.85) | | 0.000 | -16 | -100 | 10 |
| | 0.064 (40, 5.16) | 0.008 (5.16) | 0.198 | 0.000 | 44 | -48 | -26 |
| | 0.362 (33, 4.41) | 0.198 (4.41) | 0.240 | 0.000 | 18 | -72 | 48 |
| | 0.731 (35, 4.04) | 0.597 (4.04) | 0.227 | 0.000 | -32 | -48 | -20 |
| | | 0.992 (3.52) | | 0.000 | -38 | -52 | -24 |

Height threshold $\{u\} = 2.33$, $p = 0.010$
Extent threshold $\{k\} = 26$ voxels, $p = 0.296$
Expected voxels per cluster, $E\{n\} = 25.8$
Expected number of clusters, $E\{m\} = 20.6$

Volume {S} = 178896 voxels or 1642.2 Resels
Degrees of freedom due to error = 275.0
Smoothness FWHM (mm) = 12.1 11.9 6.0
{voxels} = 6.0 6.0 3.0

SPM{Z}, output of test 4



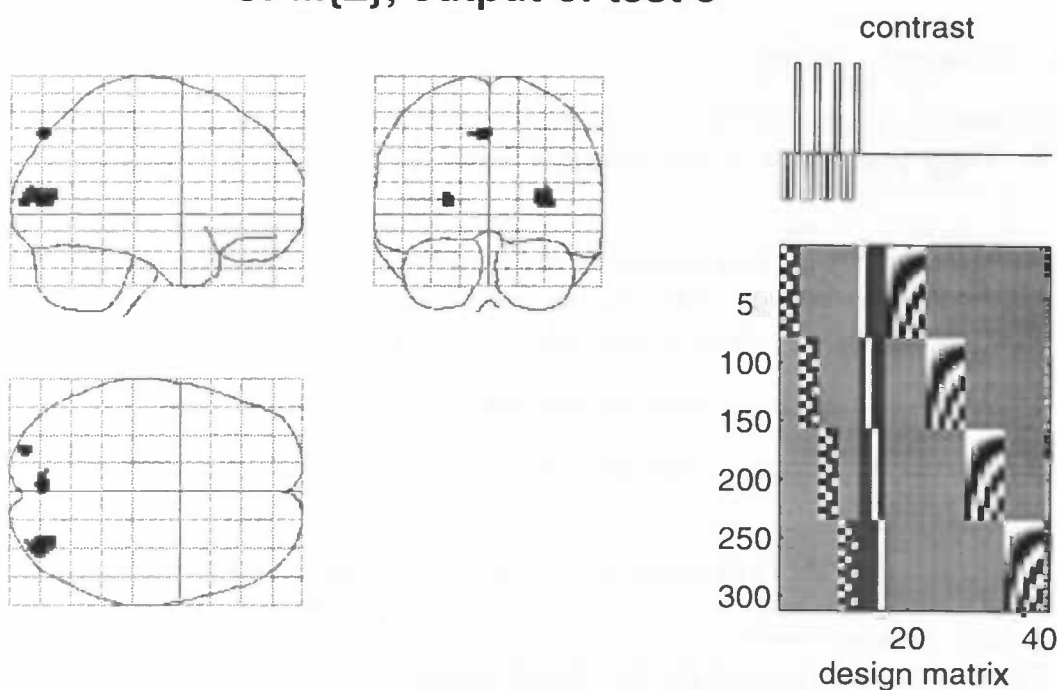
P values & statistics: ../rug106/home4/users/imaging/csg739

| set-level {c} | cluster-level {k,Z} | voxel-level {Z} | uncorrected k & Z | | x,y,z {mm} | | |
|---------------|---------------------|-----------------|-------------------|-------|------------|-----|-----|
| 1.000 (6) | 0.195 (348, 4.38) | 0.219 (4.38) | 0.001 | 0.000 | 26 | -24 | -28 |
| | | 0.370 (4.22) | | 0.000 | 28 | 0 | -26 |
| | | 0.388 (4.21) | | 0.000 | 22 | -50 | -30 |
| | 0.458 (57, 4.30) | 0.287 (4.30) | 0.128 | 0.000 | 30 | -72 | -32 |
| | | 0.806 (3.86) | | 0.000 | 52 | -48 | -32 |
| | | 1.000 (2.93) | | 0.002 | 48 | -62 | -32 |
| | | 0.673 (3.98) | 0.221 | 0.000 | 2 | -36 | -30 |
| | 0.995 (31, 3.54) | 0.989 (3.54) | 0.255 | 0.000 | -56 | 30 | -14 |
| | 1.000 (65, 3.08) | 1.000 (3.08) | 0.106 | 0.001 | 36 | -86 | 16 |
| | | 1.000 (2.82) | | 0.002 | 36 | -76 | 8 |
| | 1.000 (28, 2.99) | 1.000 (2.99) | 0.279 | 0.001 | -24 | -96 | 10 |

Height threshold {u} = 2.33, p = 0.010
 Extent threshold {k} = 26 voxels, p = 0.296
 Expected voxels per cluster, E(n) = 25.8
 Expected number of clusters, E(m) = 20.6

Volume {S} = 178896 voxels or 1642.2 Resels
 Degrees of freedom due to error = 275.0
 Smoothness FWHM {mm} = 12.1 11.9 6.0
 {voxels} = 6.0 6.0 3.0

SPM{Z}, output of test 5



P values & statistics: ../rug106/home4/users/imaging/csg739

| set-level {c} | cluster-level {k,Z} | voxel-level {Z} | uncorrected k & Z | | x,y,z {mm} | | |
|---------------|---------------------|-----------------|-------------------|-------|------------|-----|----|
| 1.000 (3) | 0.860 (33, 3.89) | 0.773 (3.89) | 0.240 | 0.000 | -4 | -80 | 48 |
| | 0.948 (120, 3.62) | 0.971 (3.62) | 0.034 | 0.000 | 32 | -82 | 10 |
| | 1.000 (26, 3.00) | 1.000 (3.00) | 0.296 | 0.001 | -24 | -90 | 6 |

Height threshold {u} = 2.33, p = 0.010
 Extent threshold {k} = 26 voxels, p = 0.296
 Expected voxels per cluster, E{n} = 25.8
 Expected number of clusters, E{m} = 20.6

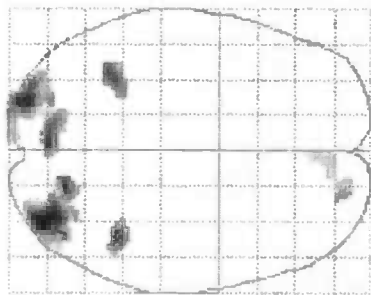
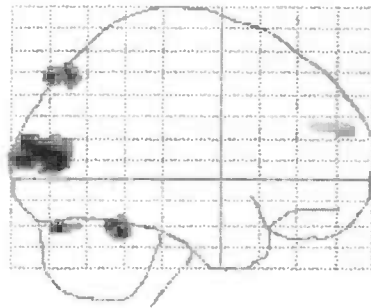
Volume {S} = 178896 voxels or 1642.2 Resels
 Degrees of freedom due to error = 275.0
 Smoothness FWHM {mm} = 12.1 11.9 6.0
 {voxels} = 6.0 6.0 3.0

5.6.1 Detailed results

For test number 1 some additional information is printed to show the potentials of SPM. The 5 pages show in the following order:

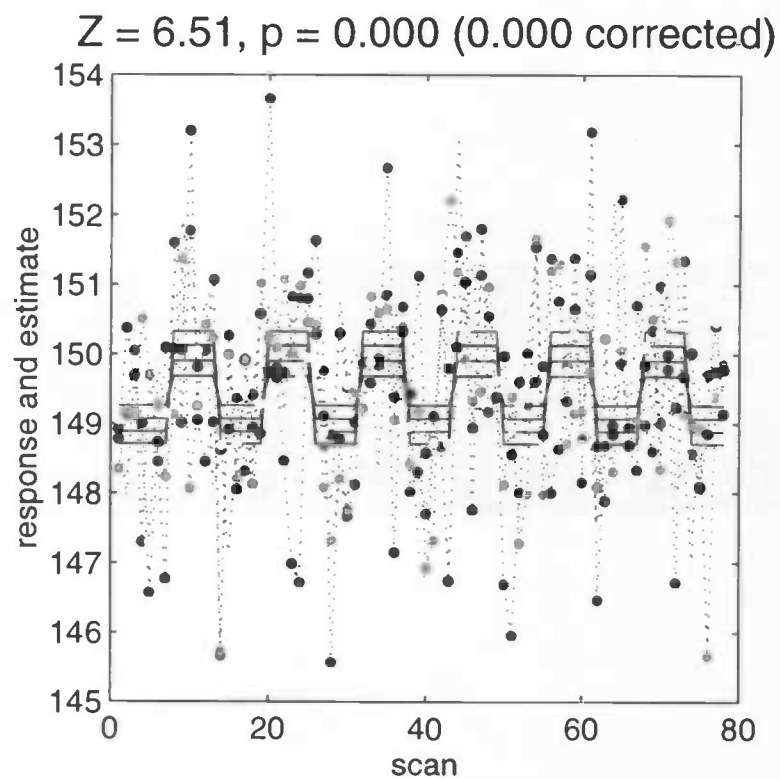
- The delayed box-car response along with an estimation of the brain-activation in an arbitrary cluster. This cluster can be chosen with the SPM-MIP (a small cursor which can be moved when running the SPM-program)
- 3 normalised functional scans around the biggest activation area.
- 3 normalised functional scans around a small activation area in the bottom of the brain.
- The orthogonal views of the functional scans, around the biggest activation area.
- The orthogonal views of the functional scans, around the small activation area in the bottom of the brain.

SPM - MIP
 $x = 36.00$
 $y = -80.00$
 $z = 10.00$

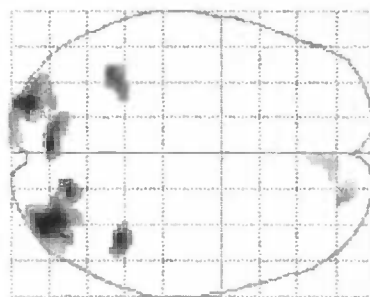
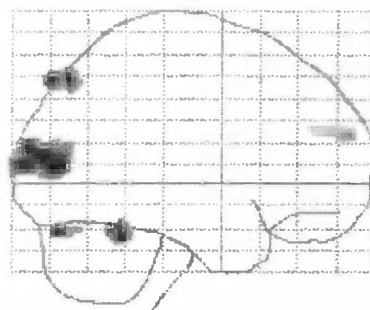


../imaging/csg739/detailed

Height threshold $\{u\} = 1.64, p = 0.050000$
 Extent threshold $\{k\} = 2.850505e+01$ voxels



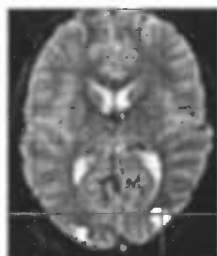
SPM - MIP
 $x = 36.00$
 $y = -80.00$
 $z = 10.00$



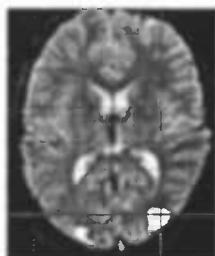
../imaging/csg739/detailed

Height threshold $\{u\} = 1.64$, $p = 0.050000$
 Extent threshold $\{k\} = 2.850505e+01$ voxels

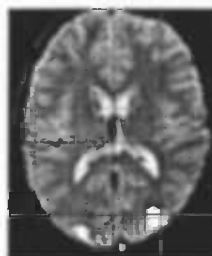
$z = 8\text{mm}$



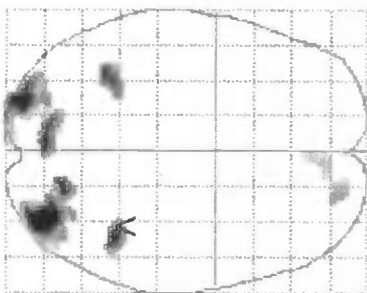
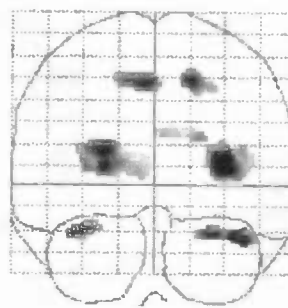
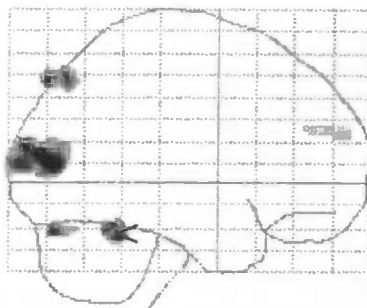
$z = 10\text{mm}$



$z = 12\text{mm}$



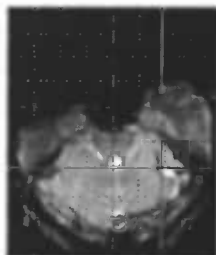
SPM - MIP
 x = 36.00
 y = -46.00
 z = -24.00



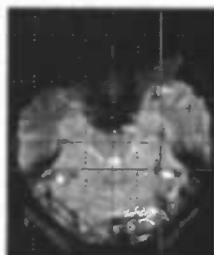
../imaging/csg739/detailed

Height threshold (u) = 1.64, p = 0.050000
 Extent threshold (k) = 2.850505e+01 voxels

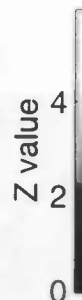
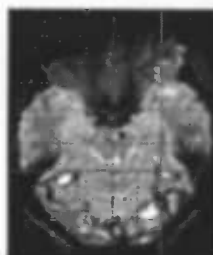
z = -26mm



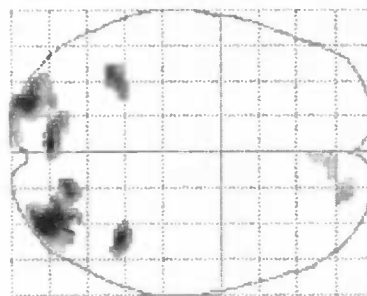
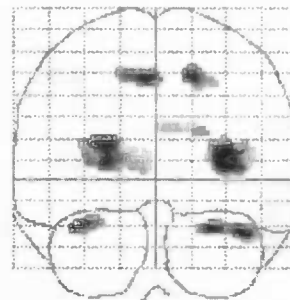
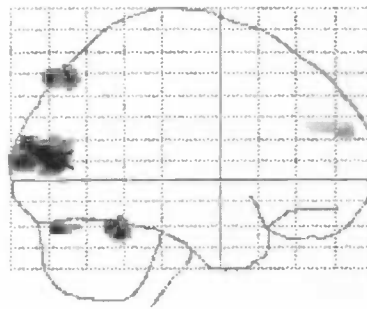
z = -24mm



z = -22mm



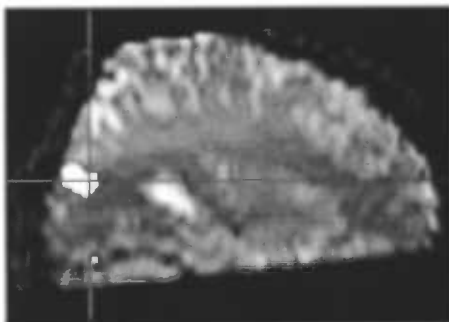
SPM - MIP
 x = 34.00
 y = -78.00
 z = 10.00



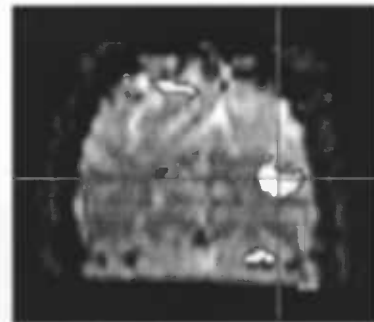
./imaging/csg739/detailed

Height threshold (u) = 1.64, p = 0.050000
 Extent threshold (k) = 2.850505e+01 voxels

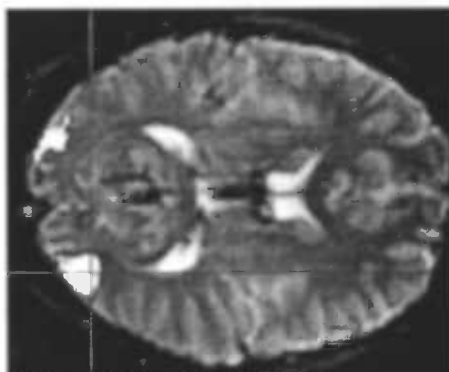
sagittal



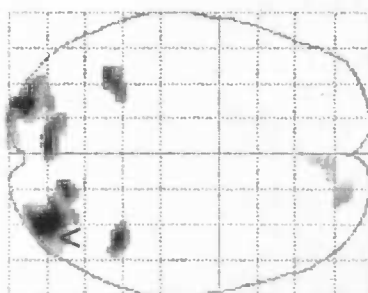
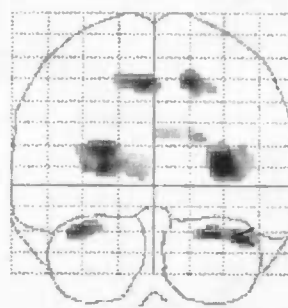
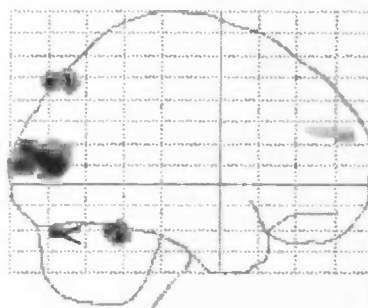
coronal



transverse



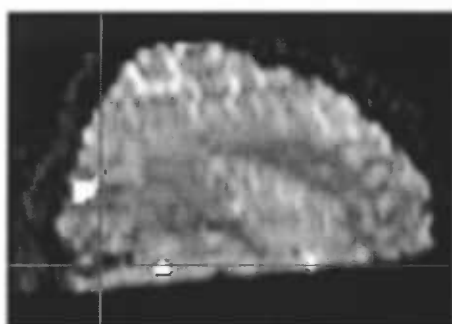
SPM - MIP
 $x = 40.00$
 $y = -74.00$
 $z = -24.00$



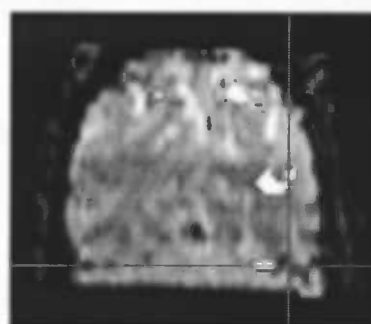
../imaging/csg739/detailed

Height threshold $\{u\} = 1.64$, $p = 0.050000$
 Extent threshold $\{k\} = 2.850505e+01$ voxels

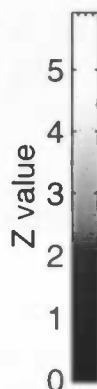
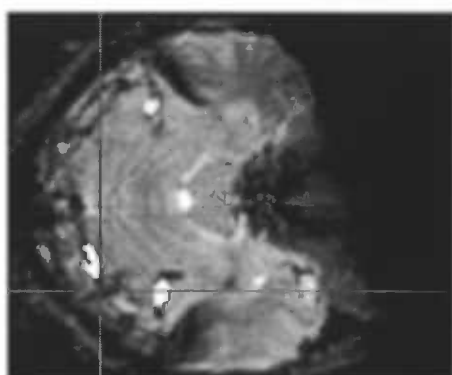
sagittal



coronal



transverse



5.7 AFNI in comparison to SPM

To have a comparison for the SPM program a secondary program called AFNI was used to compare the results of SPM with. AFNI has a different approach in analyzing files than SPM has. AFNI uses contrasts in the same way SPM does, but the output of AFNI consists of scans with activation, instead of volumes. AFNI also doesn't transform the random field to a Gaussian field.

A more serious problem is that AFNI doesn't consider the time to acquire the slices. However the data has a interscan interval of 7 seconds which should not be neglected. The current version of AFNI does not take this latency into account. Another important difference is that SPM smoothes the data before analysis, AFNI does not.

The same tests as done with SPM were performed with AFNI. The results of these tests can be seen on the following pages. For each test 12 scans are shown which lie in the lower part of the brain. The scans in top of the brain are not shown, because they show less activation.

For each scan the activation is superimposed on functional scans. Very high gray values show activation. White represents much activation.

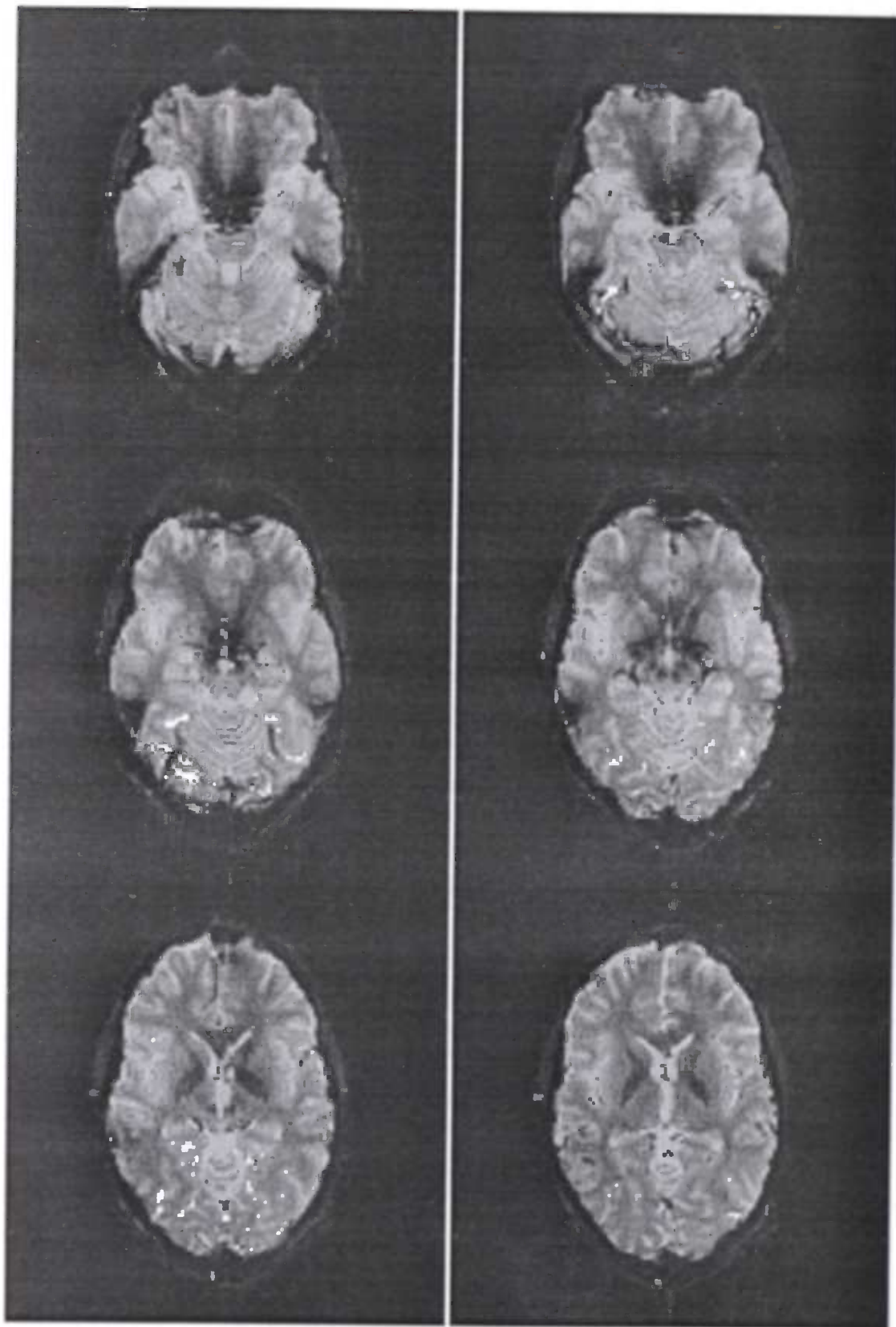


Figure 1: Testing for activation due to the memory task (alternative and normal) against the rest condition. This was done using contrast -1 1 in all of the 4 datasets. Scans 1 till 6 are shown, from topleft to bottomright. The scan with the lowest z-value is scan 0, the scan with the highest z-value is scan 24

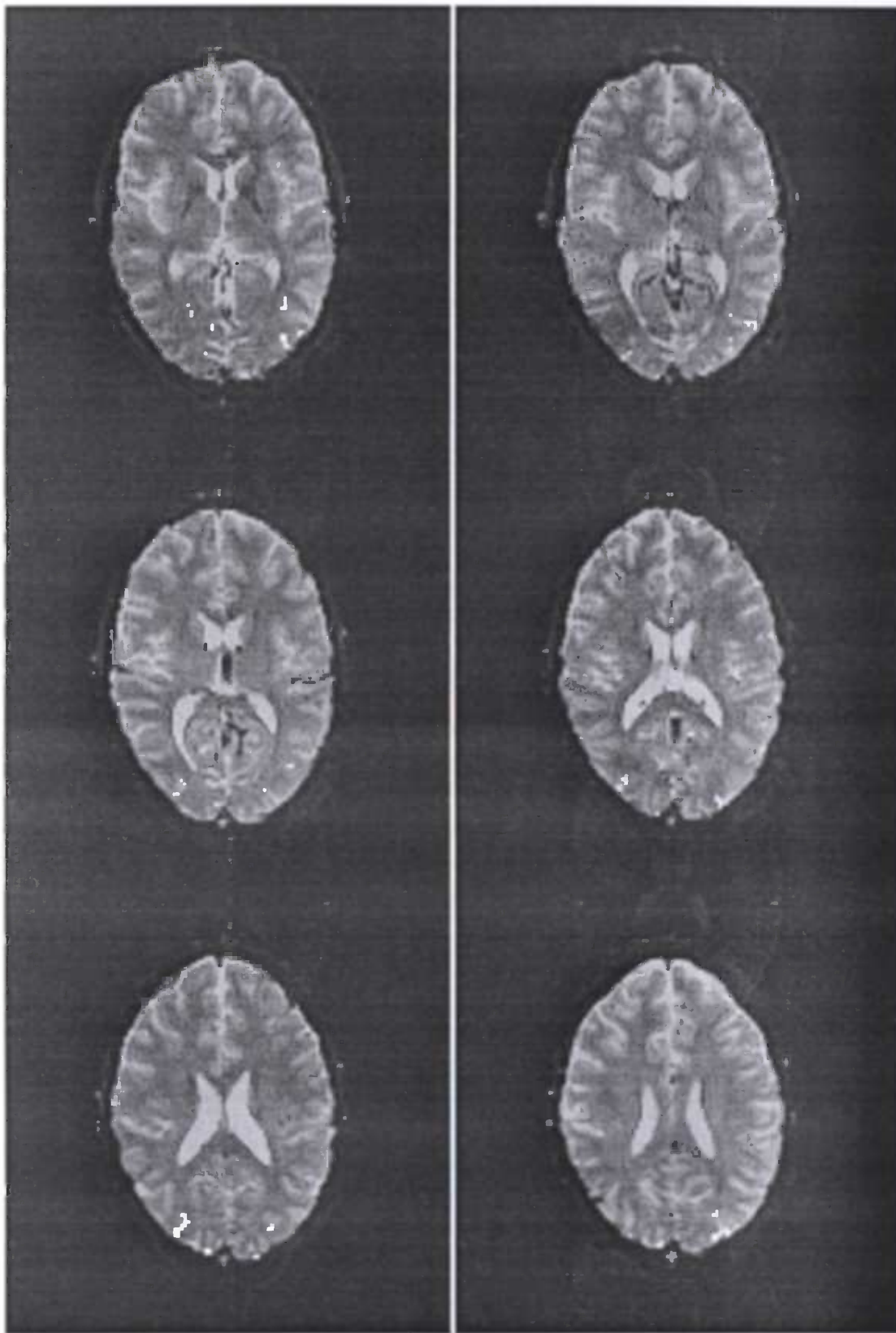


Figure 2: Testing for activation due to the memory task (alternative and normal) against the rest condition. This was done using contrast -1 1 in all of the 4 datasets. Scans 7 till 12 are shown, from topleft to bottomright. The scan with the lowest z-value is scan 0, the scan with the highest z-value is scan 24

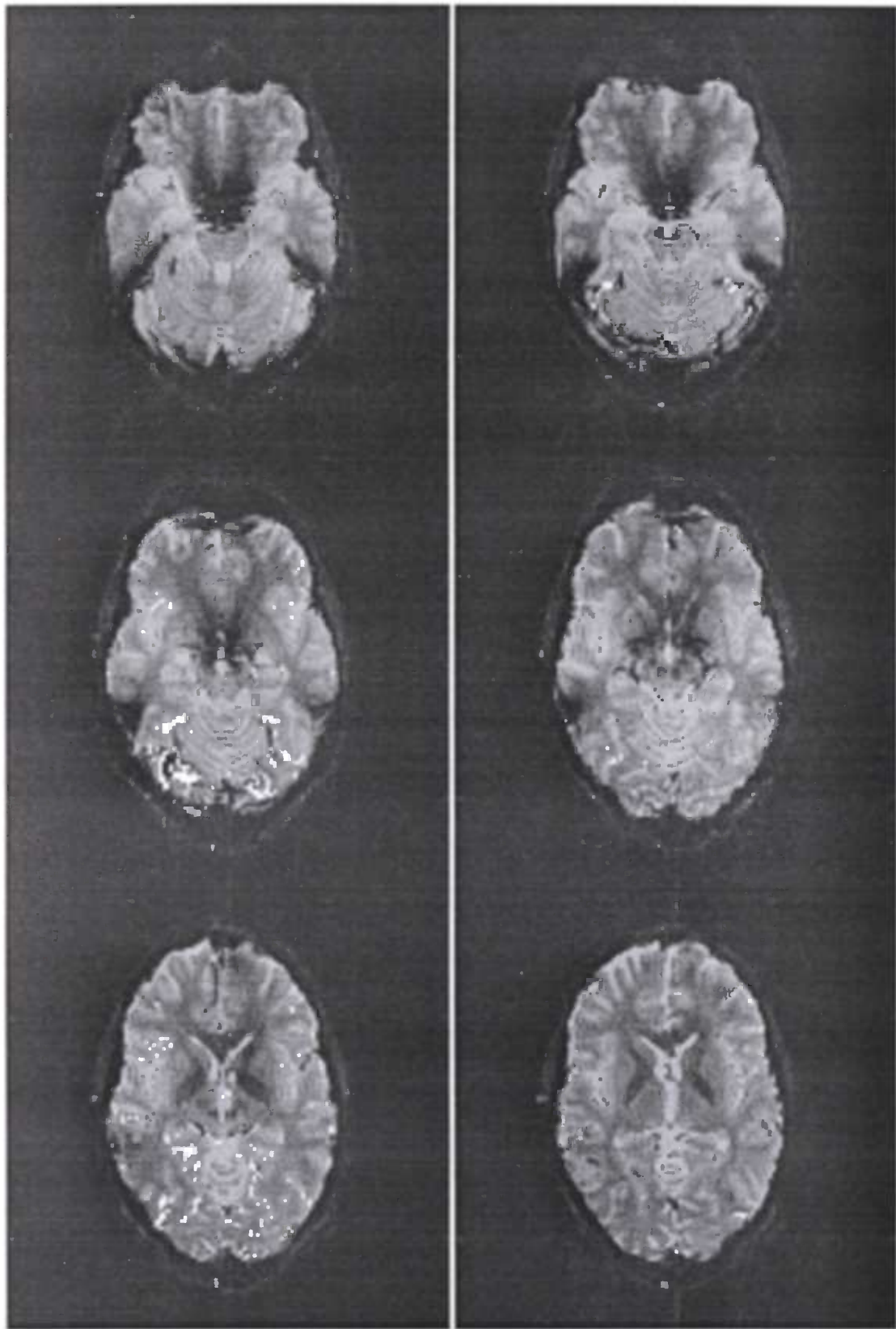


Figure 3: Testing for activation due to the normal memory task against the rest condition. This was done using contrast -1 1 0 in all of the 4 datasets. Scans 1 till 6 are shown, from topleft to bottomright. The scan with the lowest z-value is scan 0, the scan with the highest z-value is scan 24

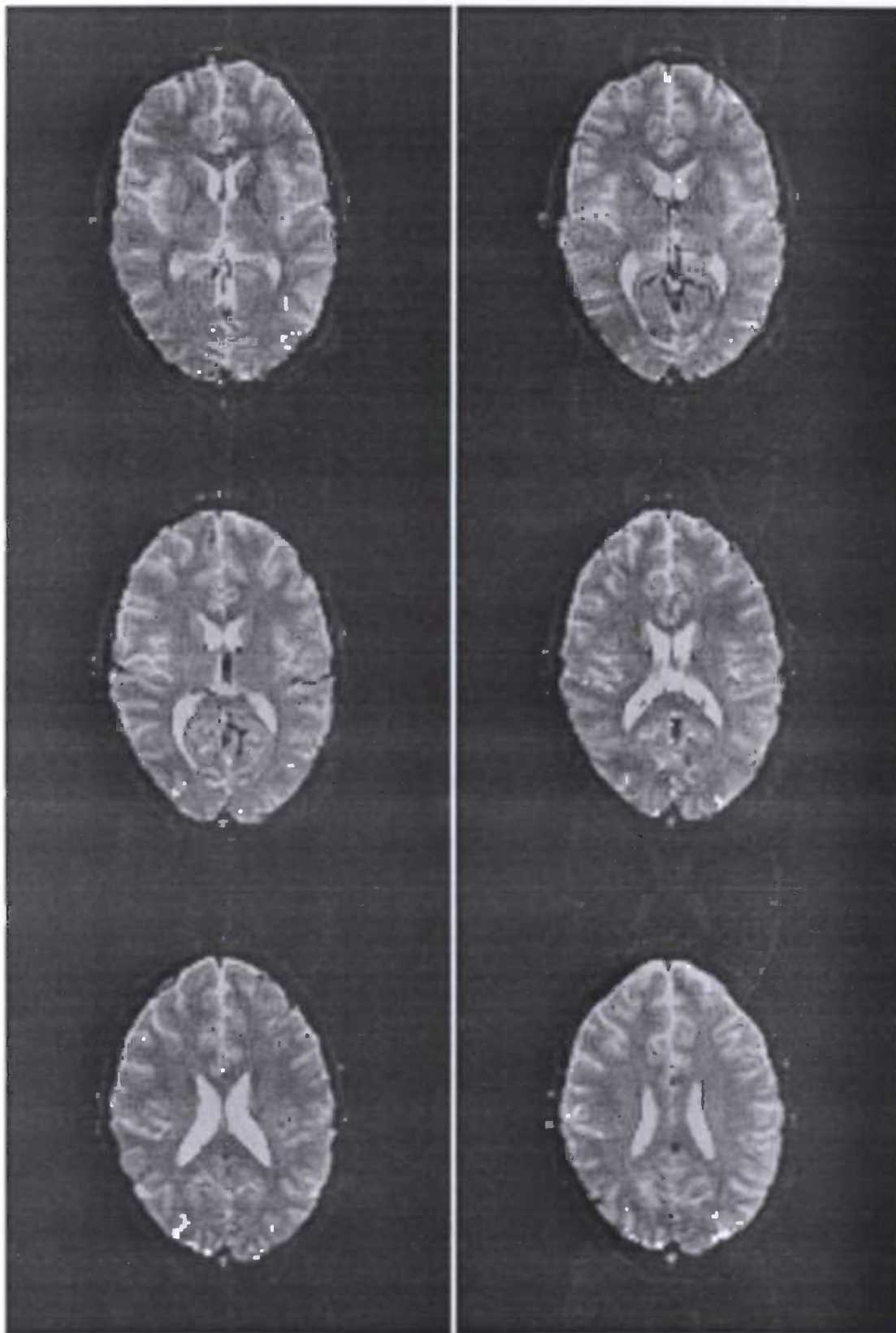


Figure 4: Testing for activation due to the normal memory task against the rest condition. This was done using contrast $-1\ 1\ 0$ in all of the 4 datasets. Scans 7 till 12 are shown, from topleft to bottomright. The scan with the lowest z-value is scan 0, the scan with the highest z-value is scan 24

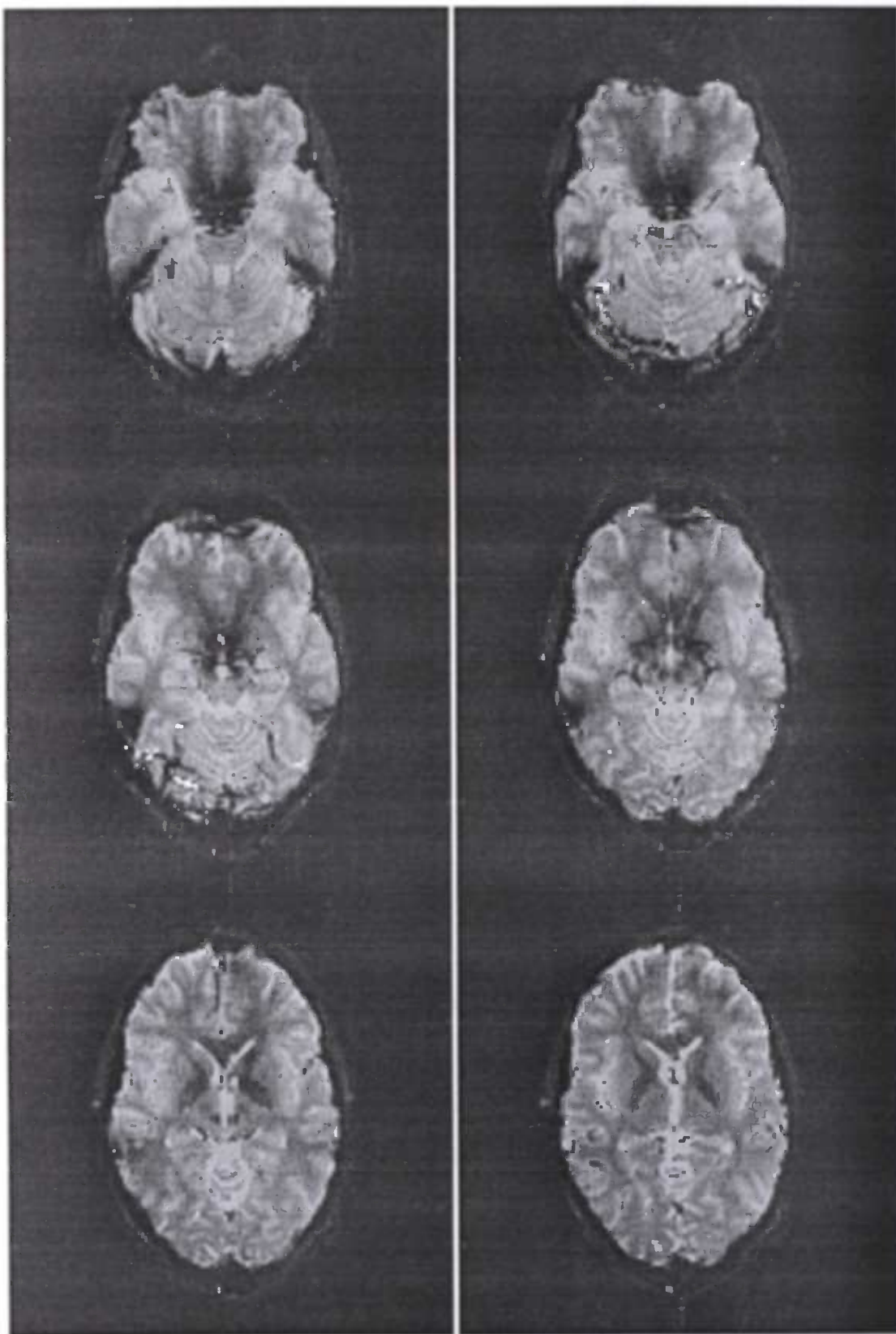


Figure 5: Testing for activation due to the alternative memory task against the rest condition. This was done using contrast -1 0 1 in all of the 4 datasets. Scans 1 till 6 are shown, from topleft to bottomright. The scan with the lowest z-value is scan 0, the scan with the highest z-value is scan 24

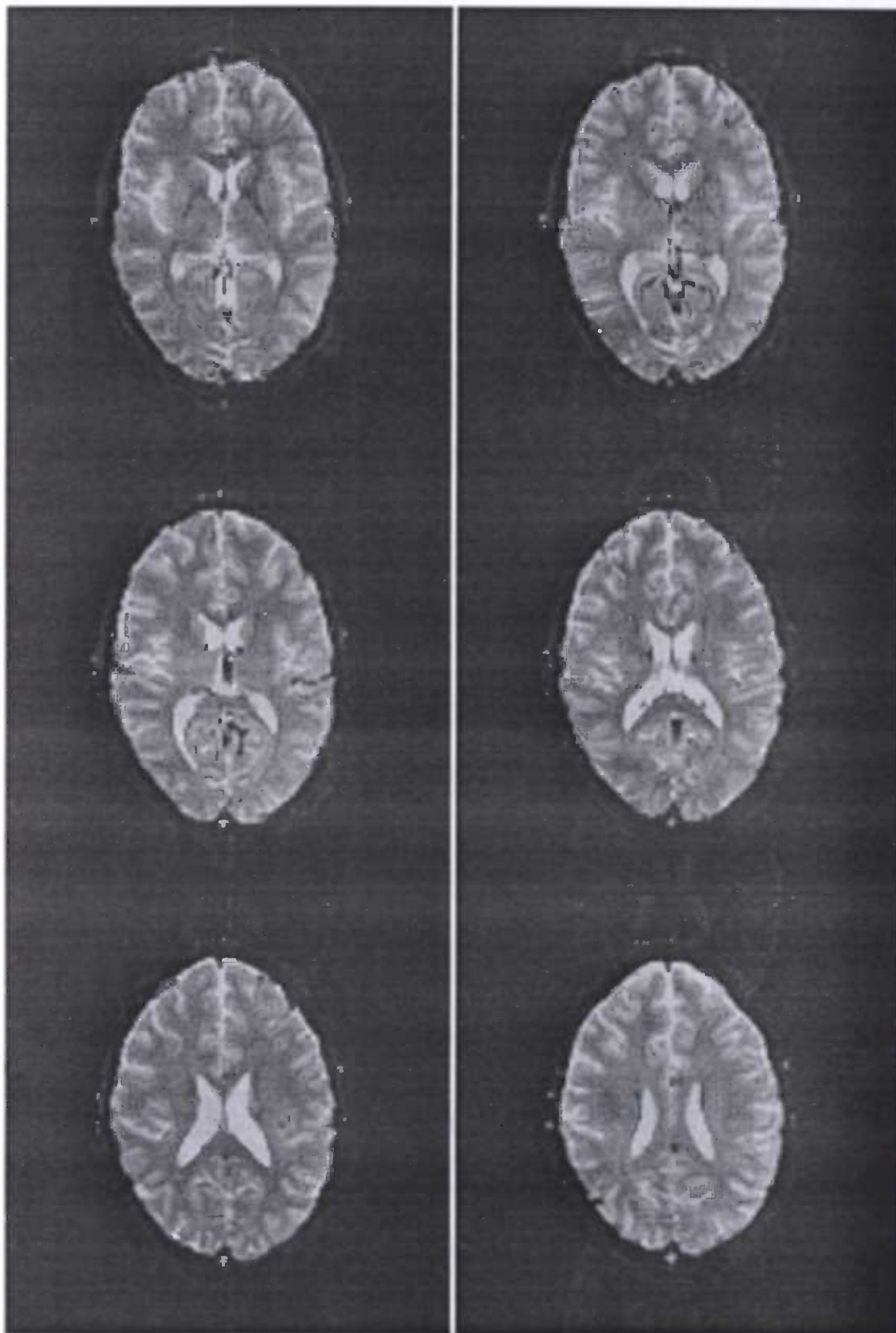


Figure 6: Testing for activation due to the alternative memory task against the rest condition. This was done using contrast -1 0 1 in all of the 4 datasets. Scans 7 till 12 are shown, from topleft to bottomright. The scan with the lowest z-value is scan 0, the scan with the highest z-value is scan 24

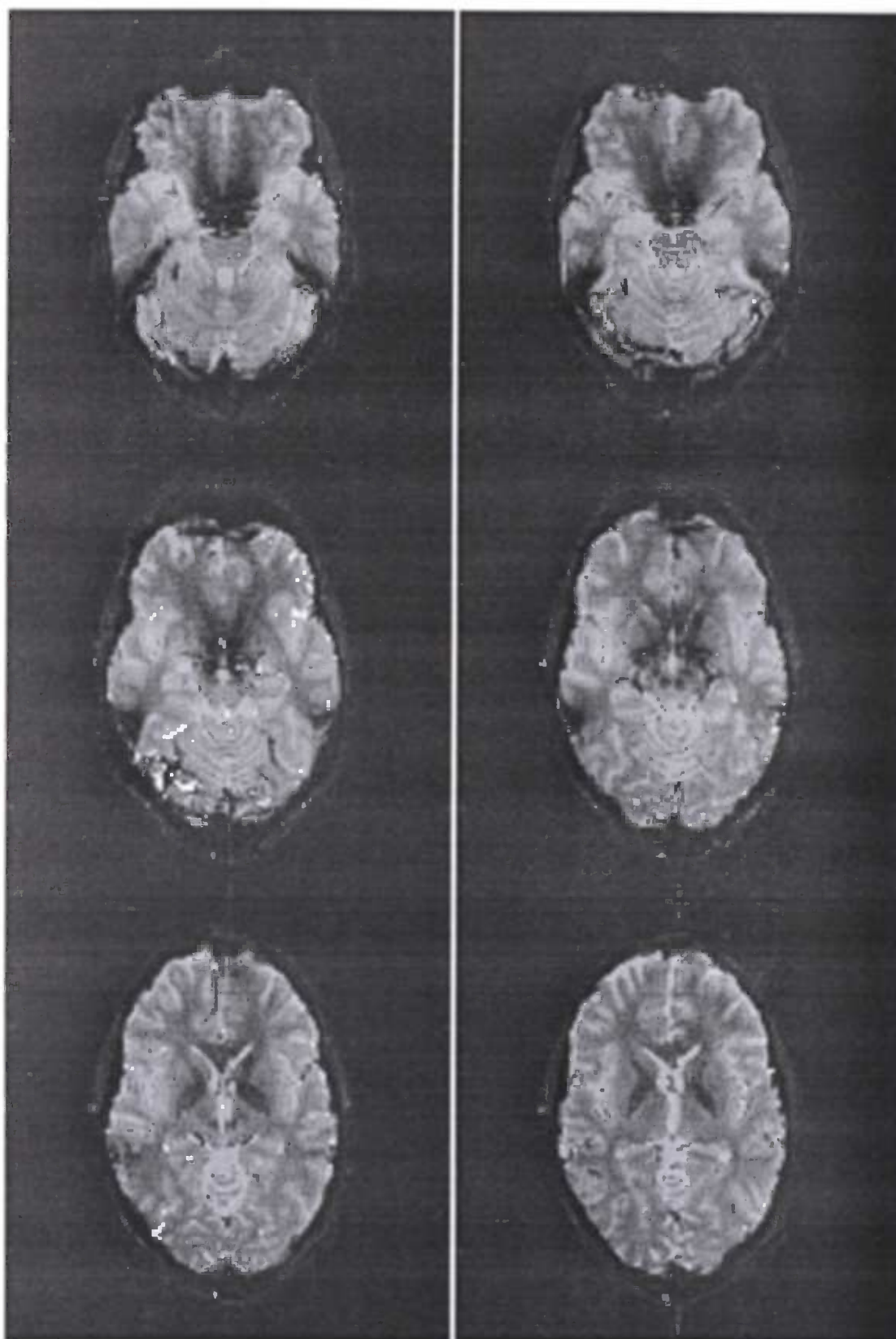


Figure 7: Testing for activation due to the memory task, without the activated areas caused by the alternative memory task. This was done using contrast -1 2 -1 in all of the 4 datasets. Scans 1 till 6 are shown, from topleft to bottomright. The scan with the lowest z-value is scan 0, the scan with the highest z-value is scan 24

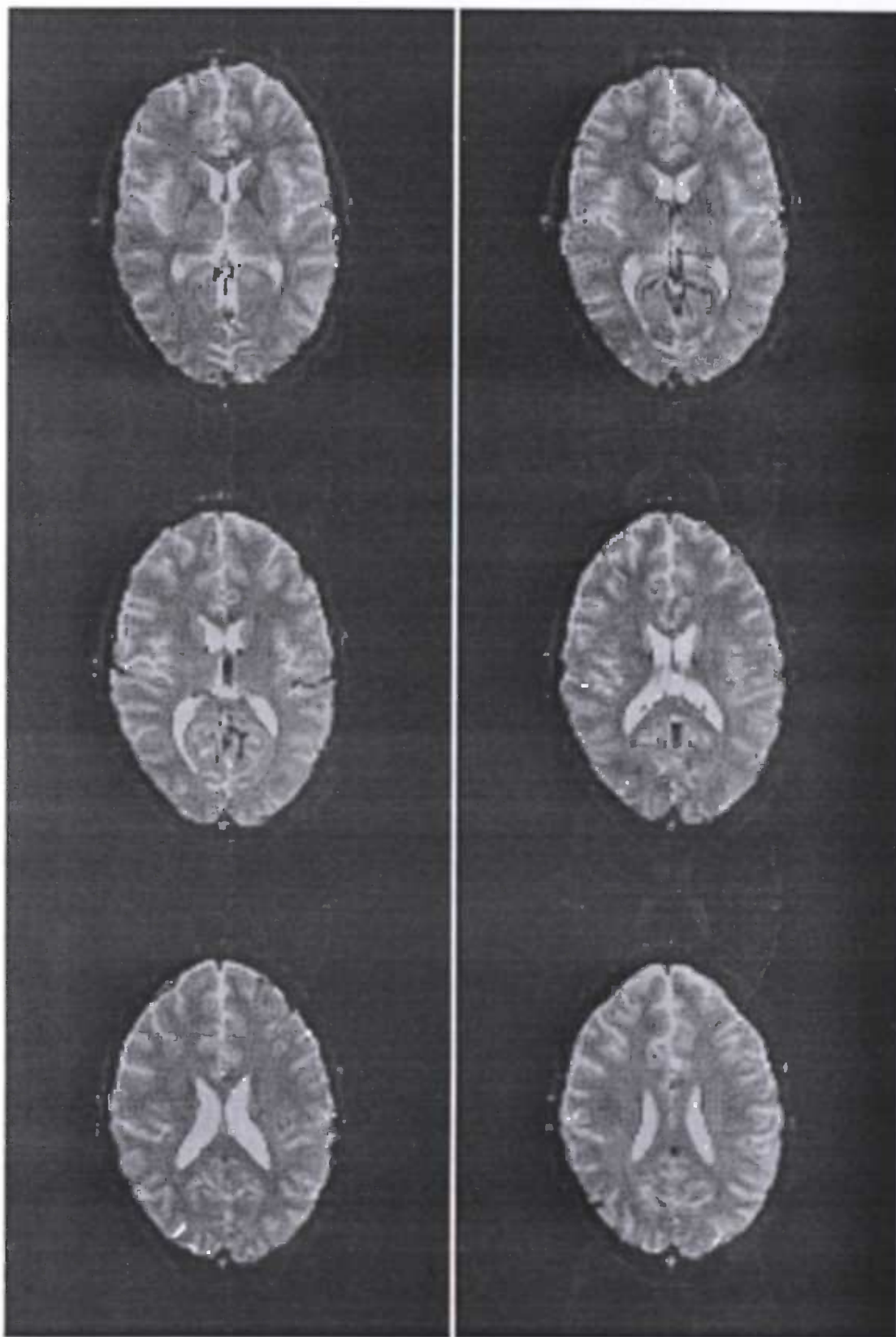


Figure 8: Testing for activation due to the memory task, without the activated areas caused by the alternative memory task. This was done using contrast -1 2 -1 in all of the 4 datasets. Scans 7 till 12 are shown, from topleft to bottomright. The scan with the lowest z-value is scan 0, the scan with the highest z-value is scan 24

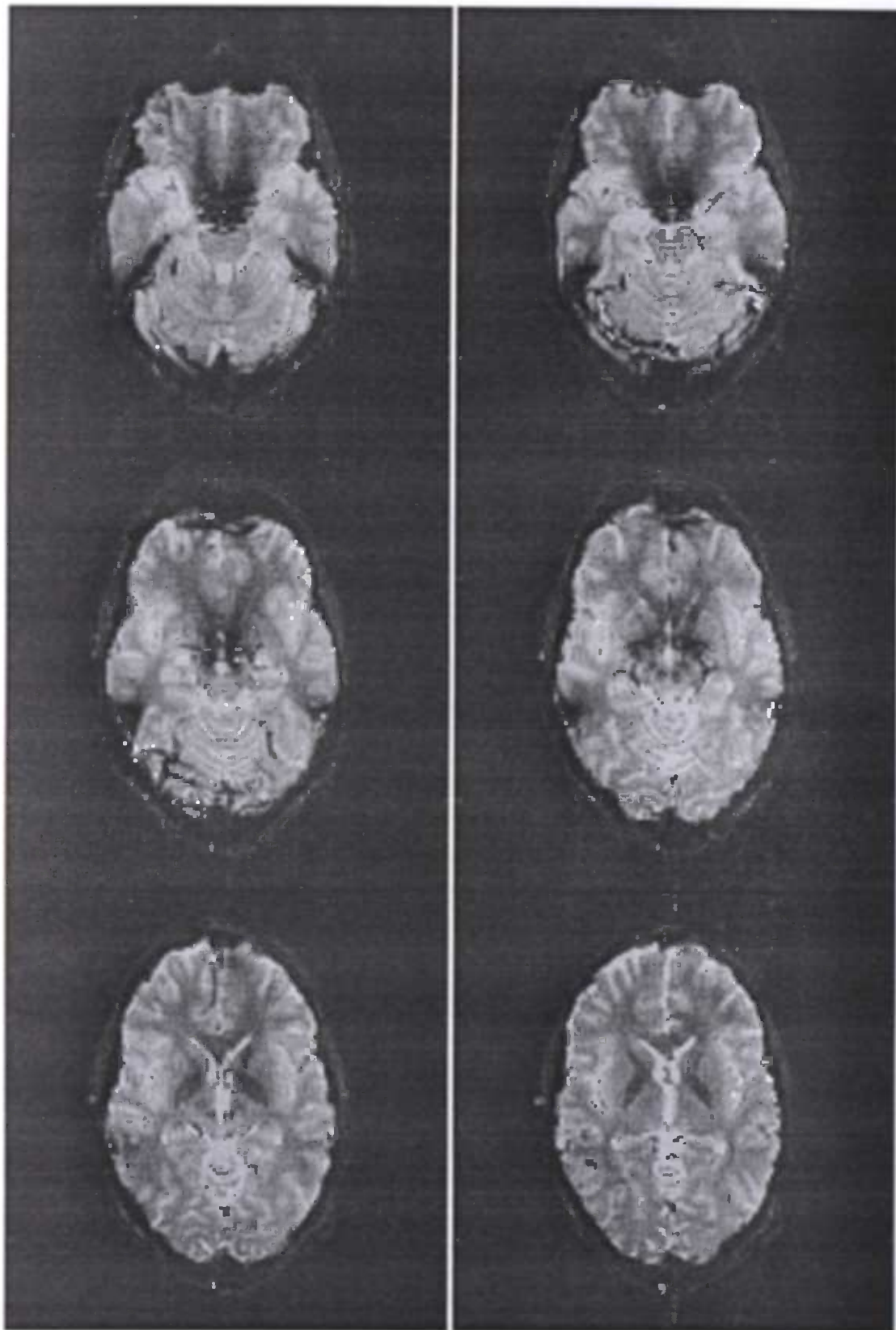


Figure 9: Testing for activation due to the alternative memory task, without the activated areas caused by the normal memory task. This was done using contrast -1 -1 2 in all of the 4 datasets. Scans 1 till 6 are shown, from topleft to bottomright. The scan with the lowest z-value is scan 0, the scan with the highest z-value is scan 24

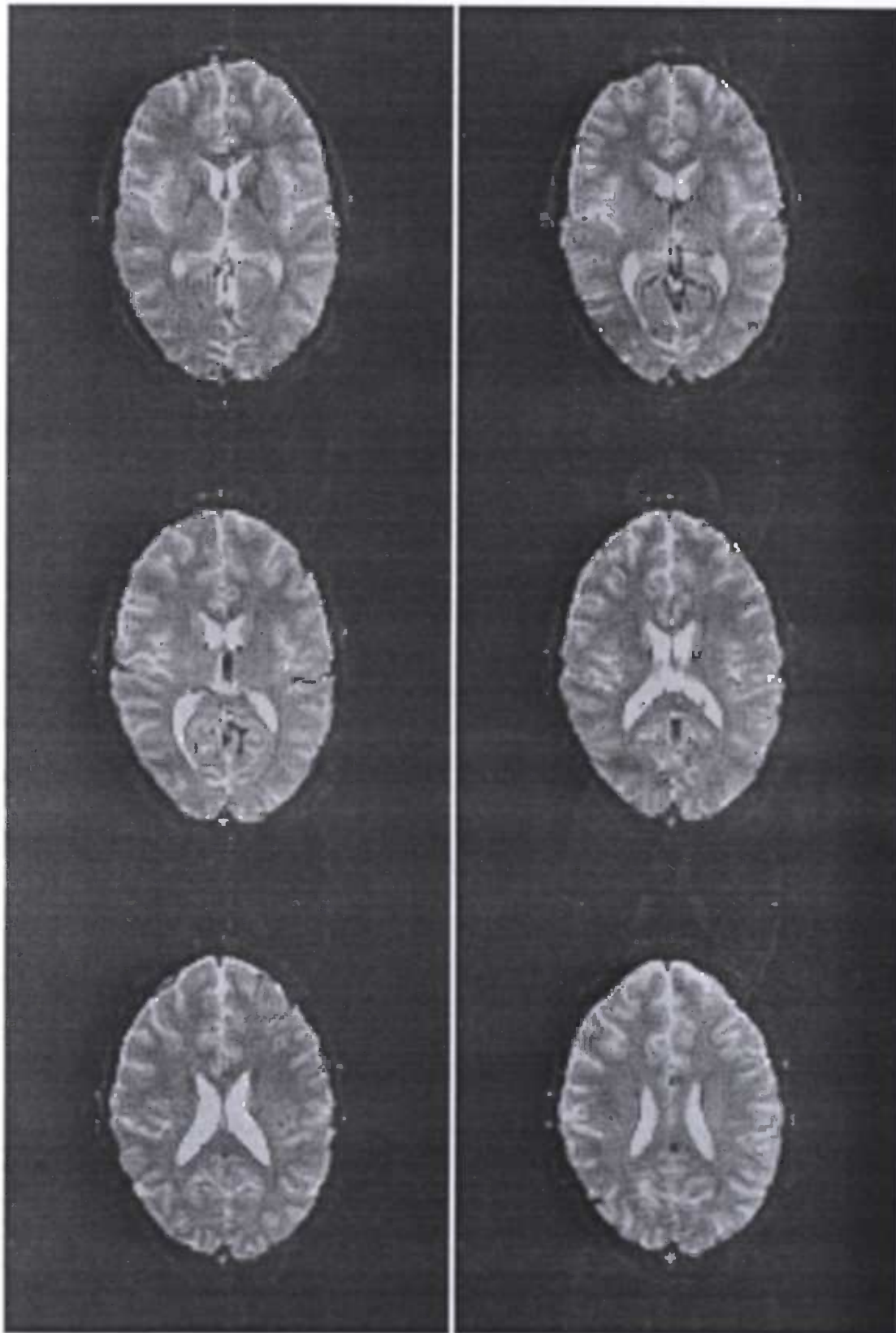


Figure 10: Testing for activation due to the alternative memory task, without the activated areas caused by the normal memory task. This was done using contrast -1 -1 2 in all of the 4 datasets. Scans 7 till 12 are shown, from topleft to bottomright. The scan with the lowest z-value is scan 0, the scan with the highest z-value is scan 24

6 Conclusions

Significant activation can be seen when looking at the results of SPM or AFNI. The contrasts which were used tested for activation. It might be a good idea to test for deactivation as well. A combination of these two is also possible in SPM, for example an activation early in the experiment followed by deactivation at the end of the experiment.

Another point to notice is that all the results depend on the selected threshold. The results might change dramatically when changing the thresholds. For this reason default p-values were used as much as possible, assuming that the authors of SPM used 'good' default p-values. Results also depend largely on the used smoothing factor. As mentioned before there is a substantial debate in the literature about the correctness of the smoothing step.

It should be verified whether the x-direction flip in the normalisation step is correct, the only way to do this is to provide some background information about the data.

The results of SPM can be compared with an independent program. The results of AFNI are shown in this thesis. A real comparison is hard to make because AFNI can't handle fMRI time-series, doesn't use a transformation to a Gaussian field and displays the activation on the scans instead of on volume images. SPM on the other hand uses smoothing which is questionable. If we however neglect these differences we see on both outputs activation in the back of the brain. SPM displays the main activation higher than AFNI does. It is difficult to give a reason for this considering the differences between the two programs.

The results were discussed with F.W. Cornelissen, who provided the data. According to his opinion the main activation shown by SPM could be seen in the **visual cortex**. Visual patterns were presented in the memory test which explains the activation in the **visual cortex**.

There are a total of 4 datasets, the datasets were used together to increase the significance of the activation. In the future, different subjects should be used to increase the validity of the activation.

Finally a comment on the layout of the experiment. The memory task can be further divided in encoding and retrieval of information in memory (see for example [17]). This might be a good idea for an experiment in the future.

A Wordlist

baseline-activation pair : Rest condition followed by activation condition.

cerebellum : A large dorsally projecting part of the brain concerned especially with the coordination of muscles and the maintenance of bodily equilibrium, situated between the brain stem and the back of the cerebrum.

cerebral : relating to the brain.

cerebral cortex : The surface layer of gray matter of the **cerebrum** that functions chiefly in coordination of sensory and motor information.

cerebrum : An enlarged anterior or upper part of the brain; especially : the expanded anterior portion of the brain that in higher mammals overlies the rest of the brain, consists of **cerebral** hemispheres and connecting structures

cortex : The outer layer of gray matter of the **cerebrum** and **cerebellum**.

cortical : Involving or resulting from the action or condition of the **cerebral cortex**.

cortico-cortical : Between two corticals.

electrophysiology : **Physiology** that is concerned with the electrical aspects of physiological phenomena.

excitation : The disturbed or altered condition resulting from stimulation.

hemodynamic : Relating to the mechanics of blood circulation.

neurophysiology : **Physiology** of the nervous system.

physiology : A branch of biology that deals with the functions and activities of life or of living matter (as organs, tissues, or cells) and of the physical and chemical phenomena involved.

psychopharmacological : Relating to the study of the effect of drugs on the mind and behaviour.

stationary fields : Fields which don't change in condition.

synapse : The point at which a nervous impulse passes from one neuron to another.

synaptic : Of or relating to a **synapse**.

visual cortex : Part of the brain responsible for processing of visual information.

References

- [1] J.R. Absher and D.F. Benson. Disconnection syndrome: an overview of Gschwind's contributions. *Neurology* 43:862-867 (1993).
- [2] R.J. Adler, "The geometry of random fields" Wiley, New York, 1981.
- [3] A. Aertsen and H. Preissl. Dynamics of activity and connectivity in physiological neuronal networks in "Non Linear Dynamics and Neural Networks" (H.G. Schuster publishers Inc., ed.)pp. 281-302. New York, USA, 1991.
- [4] J. Ashburner, P. Neelin, D.L. Collins, A. Evans, K. Friston Incorporating prior knowledge into image registration. *Neuroimage*, 6(4):344-352, 1997.
- [5] C. Chatfield and A.J. Collins Introduction to multivariate analysis (1980). Chapman and Hall, London, 189-210.
- [6] Robert W. Cox AFNI version 2.21: Copyright Medical College of Wisconsin: November 1998. Available at <http://varda.biophysics.mcw.edu/~cox/>
- [7] W.F. Eddy, M. Fitzgerald, D.C. Noll. Improved image registration by using Fourier interpolation (1996). *Magnetic resonance in medicine*, 36, 923-931.
- [8] Karl J. Friston. Basic concepts and overview: SPM course May 1997 (chapter 1).
- [9] K.J. Friston, J. Ashburner, C.D. Frith, J.-B. Poline, J.D. Heather, R.S.J. Frackowiak. Spatial registration and normalization of images. (1995) *Human brain mapping*, 2, 165-189.
- [10] K.J. Friston, C.D. Frith, R.S.J. Frackowiak. Time-dependent changes in effective connectivity measured with PET. *Human Brain Mapping* 1:69-80 (1993).
- [11] K.J. Friston, C.D. Frith, P.F. Liddle, R.S.J. Frackowiak. Functional connectivity: the principal component analysis of large (PET) data sets. *J. Cereb. Blood Flow Metab.* 13:5-14 (1993).
- [12] K.J. Friston, P. Grasby, C. Bench, C. Frith, P. Cowen, P. Little, R.S.J. Frackowiak, R. Dolan. Measuring the neuromodulatory effects of drugs in man with positron tomography. *Neuroscience Letters* 141:106-110 (1992).
- [13] K. Friston, A.P. Holmes, K. Worsley, J.-B. Poline, C. Frith and R.S.J. Frackowiak (1995). Statistical parametric maps in functional imaging: A general linear approach. *Human Brain mapping* 2, 189-210.

- [14] K.J. Friston, K.J. Worsley, R.S.J. Frackowiak, J.C. Mazziotta, A.C. Evans. Assessing the Significance of Focal Activations Using their Spatial Extent(1994). *Human Brain Mapping*, 1, 214-220.
- [15] A.M. Hasofer Upcrossings of random fields(1978) *Suppl. Adv Appl. Prob.* 10:14-21
- [16] A.M. Howseman, O. Jesechs, G. Rees and K.J. Friston. Special Issues in Functional magnetic Resonance Imaging. SPM course May 1997 (chapter 9).
- [17] J.D.E Gabrieli, J.B. Brewer, J.E. Desmond and G.H. Glover. Separate neural bases of two fundamental memory processes in the human medial temporal lobe. *Science* 1997;276.264-266.
- [18] G.L. Gerstein, P. Bedenbaugh, A.M.H.J. Aertsen. Neuronal assemblies *IEEE. Trans. on Biomed. Engineering* 36:4-14 (1989).
- [19] F. Goltz. Transactions of the 7th international medical congress (W MacCormac, ed) Vol. I, pp. 218-228. JW Kolkman: London 1881.
- [20] S.Grafton, J. Mazziotta, S. Presty, K.J. Friston, R.S.J. Frackowiak, M. Phelps. Functional anatomy of human procedural learning determined with regional cerebral blood flow and PET. *J. Neuroscience* 12:2542-2548 (1992).
- [21] P. Grasby, K.J. Friston, C. Bench, P. Cowen, C. Frith, P. Liddle, R.S.J. Frackowiak, R. Dolan. Effects of 5-HT1A partial agonist buspirone on regional cerebral blood flow in man. *Psychopharmacology* 108:380-386 (1992).
- [22] J.I. Nelson, P.A. Salin, N.M.J. Munk, M Arzi, J. Bullier. Spatial and temporal coherence in cortico-cortical connections: a cross-correlation study in areas 17 and 18 in the cat. *Visual Neurosci.* 9:21-37 (1992).
- [23] Erik Nolf medcon 3.4
Available at <http://petaxp.rug.ac.be/~nolf/welcome.html#CONVERSION>
- [24] C.G. Phillips, S. Zeki, H.B. Barlow. Localization of function in the cerebral cortex. Past, present and future. *Brain* 107:327-361(1984).
- [25] W.H. Press, S.A. Teukolsky, W.T. Vetterling, B.P. Flannery. Numerical recipes in C (second edition) (1992) Cambridge university Press. Cambridge.
- [26] J.A. Rice. Mathematical Statistics and Data Analysis, second edition. Duxbury Press (1995), 323.
- [27] J. Talairach & P. Tournoux. A stereotactic coplanar atlas of the human brain. Stuttgart: Thieme (1988).

- [28] Roger P. Woods AIR3.08. Department of Neurology UCLA School of Medicine.
Available at <http://bishopw.loni.ucla.edu/AIR3/download.html>

