COMPARISON OF DIFFERENT STATISTICAL ANALYSES IN VISUAL STIMULUS FMRI

M. PEDERSEN (1, 2), F.A. BARRIOS (1, 3)

(1) Imagerie Moléculaire et Fonctionnelle : de la Physiologie à la Thérapie, Université Victor Segalen Bordeaux 2, Bordeaux, France.
(2) MR Research Center, Clinical Institute, Aarhus University Hospital, Skejby, 8200 Aarhus N, Denmark.
(3) Instituto de Neurobiologia, UNAM, Campus Juriquilla UNAM, 76230 Queretaro, QRO Mexico.

INTRODUCTION

Functional magnetic resonance imaging (fMRI) is now an established method for studying the functional anatomy of the brain. fMRI is becoming increasingly available to investigate the visual cortex. However, one major problem makes the detection of neural activity difficult: the noisy character of the fMRI data. This means that simple image subtraction between activated and non-activated pixels is usually inadequate for reliable registration of the neural activity. Instead, the most widely used method to detect neural activity is cross-correlation analysis or the Student’s t-test, and the result is a scattered activation pattern. These methods register each pixel independently of its neighbours with no regard for its spatial context. Nonetheless, the different statistical methods applied could generate different results, leading to different conclusions about the underlying activity of the brain.

The purpose of t-test statistics is to define a resting state baseline and to compare the images acquired at each point before, during, and after the stimulus with this baseline. Then, calculation of the t score generates a set of t-statistical parametric maps. On the other hand, correlation analysis is based on a correlation between a time course and a reference waveform using a normalized cross correlation coefficient [15] that has a value of 1 for perfect correlation, a value of 0 for no correlation, and a value of –1 for perfect anticorrelation. Obviously, the choice of an appropriate reference waveform is vital for successful analysis. Moreover, the calculated correlation coefficient can be transformed so that it has a Gaussian distribution with zero mean and unit variance (also called the Z distribution), allowing Z statistical parametric maps to be derived with the activated pixels having a Z score above a certain threshold. We refer to previous work [1, 8, 10] for more in-depth details about statistical methods in fMRI.

This study attempts to characterize the fMRI response both of the whole brain and the visual cortex in response to a visual stimulus using four different standard statistical approaches (Z statistics, Student’s t-test, Spearman’s correlation analysis, and normalized cross-correlation analysis). As reference
standard, the Z cluster statistics was used. As a measure of neural activation, the total number of activated pixels was counted and compared among methods.

METHODS AND MATERIALS

Eight healthy adult volunteers without neurological deficits were recruited for the study. All gave written informed consent according to our institutional guidelines.

MR Imaging

MR imaging was performed on a clinical Philips Intera 1.5 Tesla system (Philips Medical Systems, Best, Netherlands) equipped with a maximum gradient strength of 23mT/m and a slew rate of 120mT/m/s. A cylindrical quadrature 6-channel radio-frequency head-coil was used for both transmission and reception. Prior to functional studies, high-resolution T2-weighted images were acquired for anatomical localization using the spin-echo parameters: TR=6,902ms, TE=100ms, 30 slices with a thickness of 2mm with zero gap, quadratic field-of-view (FOV) of 256mm, and an acquisition matrix of 256×256. fMRI was performed using the BOLD contrast method as an indirect marker of neural function. This was conducted using a T2*-weighted gradient-echo scheme with a single-shot echo-planar-imaging free induction decay (EPI-FID) pulse sequence in positions identical to those of the anatomical reference images. Acquisitions were accelerated using SENsitivity Encoding (SENSE) with a SENSE reduction factor of 2.0 [14]. The imaging parameters of the EPI-FID sequence were: TR=2,635ms, TE=50ms, 30 slices, thickness=4mm, FOV=256mm with a rectangular readout coverage of 80% and an acquisition matrix of 64×48×30, resulting in a 4×4×4mm³ isotropic resolution. In addition, a prepulse was applied for fat-suppression. A total of 120 images was dynamically acquired giving an acquisition time of 271 seconds per functional study. Shimming was performed prior to each echo-planar acquisition time of 271 seconds per functional study.

Perception stimulus and paradigm

The visual stimulus consisted of a checkerboard black-and-white box car pattern flashing at a rate of 8Hz and with a red cross in the center as the fixation point. The paradigm was presented in on-off blocks during 10 dynamic acquisitions, where off-periods presented a dark screen with the fixation point only, and on-periods presented the flashing checkerboard. The pattern was generated by Presentation (Neurobehavioral Systems, Albany, CA) and projected via a 21” MR-compatible monitor (Philips Medical Systems, Best, Netherlands).

Image analysis of fMRI data

Acquired fMRI data were converted from Philips format to ANALYZE (AnalyzeDirect Inc, Lenexa, KS) format writing an image header using the Averreadeh-tool from FSL (FMRI Expert Analysis Tool, part of FSL Version 5.0, FMRIB Software Library, John Radcliffe Hospital Oxford, United Kingdom; http://www.fmrib.ox.ac.uk/fsl) and were subsequently analyzed by two different approaches. FSL functional analysis tools were used. First, the default procedure within the FEAT software was used. Initially, motion correction was employed using the MCFLIRT routine, where a three-dimensional image registration routine was applied to the volumes for realignment with the first volume of the first series used as a spatial reference. In a second step, a spatial smoothing was conducted to account for residual intersession differences using a Gaussian kernel with a full-width-half-maximum (FWHM) of 5mm. Functional time-series statistical analysis with a box car response type was performed using FILM (FMRIB Improved Linear Model; FMRIB Centre, Department of Clinical Neurology, University of Oxford, United Kingdom) with a local autocorrelation correction (Z cluster statistics), and images were calculated with a Z>2.3 threshold and a corrected cluster significance of P=0.01 [6].

In addition, functional analyses were performed using 3 other statistical approaches: the t-test method, cross-correlation, and the Spearman correlation test. Motion corrected and normalized data in ANALYZE formatted files were transferred to the MISTar software package (Apollo Medical Technology, Melbourne, Australia). As a first step, data were spatially smoothed by convolution with a three-dimensional Gaussian function (FWHM=5mm). The dynamically constructed matrices were subsequently realigned using the first as a reference and adjusted for residual motion-related signal changes. Then, a box–car response type was used to model an actual BOLD response, resembling the original activation paradigm of the study. The box car pattern was convolved with a Gaussian function to account for hemodynamic effects. Three different statistical approaches were used to generate functional parameter maps:

A Student’s t-test analysis was performed with a t score threshold corresponding to a confidence level of P<0.001.

A nonparametric Spearman’s correlation analysis [1] was performed. After transformation of Spearman’s correlation factors into t statistics, pixels were considered activated at a threshold corresponding to a significance level of P<0.001.

Time series cross-correlation analysis was performed in which pixel-by-pixel normalized cross-correlation coefficients were calculated by matching the fMRI signal time course to the stimulus paradigm. An activation threshold with correlation coefficient of r=0.3 (P<0.01) was used [4].

For visualization, color-coded quantitative maps of positive contrasts were superimposed on the corresponding T2-weighted templates. Calculated parametric maps based on Z statistics, Student’s t-test, Spearman’s correlation, and cross-correlation were compared using a simple pixel counting method. The functional maps were first converted to 1-bit masked images using a common threshold. Neural activation was subsequently assigned to pixels having a value of 1, whereas all other pixels had a value of 0. Areas within the whole brain and the visual cortex were
identified using Talairach coordinates and anatomical landmarks [18], and the number of activated pixels was determined for each slice. Next, the number of total pixels for each slice was summed, giving a total-number-of-activated-pixels (TNAP) for the visual cortex and the whole brain. The TNAPs for the Student’s t-test, Spearman’s correlation, and cross-correlation were statistically compared with the TNAP obtained by the Z cluster statistics using two-tailed paired t-test statistics. Data are presented as mean ± standard error of mean. P<0.05 was considered to be statistically significant.

RESULTS

In all eight subjects, activation was detected in the expected areas of the visual cortex in response to the on-off paradigm. In all subjects, an increase in signal intensity, a peak, and then a decrease in signal intensity were observed in these areas. The size of activation in the visual cortex, however, was different for each subject as demonstrated previously by Rombouts [16], indicating that the selected threshold to obtain a neural activation of comparable size and localization may vary among subjects. The functional activation in response to the visual stimulus was demonstrated with Z statistics (figure 1a), Student’s t-test (figure 1b), Spearman’s correlation (figure 1c), and cross-correlation (figure 1d). Surprisingly, areas beyond the visual cortex were found to be active and are likely to be involved in the perceptual discrimination of the on-off visual stimulus, especially those in the frontal regions.

The TNAP of each subject and each statistical method is given both for the whole brain (table I) and for the visual cortex (table II), and the TNAP data are shown graphically in figure 2 as a relative index using TNAP measured with the Z statistics as reference. The indexed TNAP of the visual cortex demonstrated no significant difference as compared with Z statistics (Student’s t-test: 91±4%, p>0.05; Spearman’s correlation: 97±10%, p>0.05; cross-correlation: 93±10%, p>0.05). Likewise the indexed TNAP of the whole brain was insignificantly different from Z statistics (Student’s t-test: 102±8%, p>0.05; Spearman’s correlation: 132±10%, p>0.05), whereas a significant difference was observed using the cross-correlation method (142±15%, p=0.01).

DISCUSSION

The outcomes of brain function analyses using an inherently indirect method such as fMRI are largely derived from visual observations of activation clusters, which limit most studies of function-mapping objectives. However, the lack of unique and well-defined statistical methods makes the quantification of the neural activity fairly ambiguous. Nevertheless, as a convenient estimation of a figure-of-merit of the actual activity, we chose the number of pixels above a certain threshold, which is often used as a measure of the spatial functional activity in response to an applied stimulus. Such number of statistically chosen pixels may be critically dependent on the choice of statistical method; the objective of this work was, therefore, to compare different statistical strategies for the analysis of fMRI data, keeping in mind that all these statistical maps represent only a figure-of-merit of the neural activation and not a direct measure of the actual activation.

Findings of the present study demonstrated that neural activations detected in the parametric statistical maps were all in good agreement with previous findings, where an increase in signal intensity, a peak, and then a decrease in signal intensity could be observed in the visual cortex of the brain [5, 11, 12, 19, 20]. Unfortunately, there is not yet a consensus on how to statistically quantify changes in brain activation or how to compare the results obtained under different tasks or groups of subjects. It is therefore important to emphasize that the identification of activated pixels relies on the threshold, which can be

<table>
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<tr>
<th>TNAP/Subject</th>
<th>#1</th>
<th>#2</th>
<th>#3</th>
<th>#4</th>
<th>#5</th>
<th>#6</th>
<th>#7</th>
<th>#8</th>
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<tr>
<td>Z statistics (reference)</td>
<td>236</td>
<td>380</td>
<td>626</td>
<td>201</td>
<td>325</td>
<td>521</td>
<td>542</td>
<td>15</td>
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<tr>
<td>Student’s t-test</td>
<td>266</td>
<td>349</td>
<td>629</td>
<td>203</td>
<td>234</td>
<td>520</td>
<td>587</td>
<td>19</td>
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<tr>
<td>Cross-correlation</td>
<td>418</td>
<td>526</td>
<td>590</td>
<td>247</td>
<td>398</td>
<td>599</td>
<td>702</td>
<td>21</td>
</tr>
<tr>
<td>Spearman’s correlation</td>
<td>430</td>
<td>509</td>
<td>601</td>
<td>270</td>
<td>404</td>
<td>642</td>
<td>701</td>
<td>29</td>
</tr>
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</table>

TABLE II. – The total-number-of-activated-pixels (TNAP) of the whole brain.

<table>
<thead>
<tr>
<th>TNAP/Subject</th>
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<th>#2</th>
<th>#3</th>
<th>#4</th>
<th>#5</th>
<th>#6</th>
<th>#7</th>
<th>#8</th>
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<tr>
<td>Z statistics (reference)</td>
<td>157</td>
<td>261</td>
<td>443</td>
<td>99</td>
<td>223</td>
<td>376</td>
<td>498</td>
<td>12</td>
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<tr>
<td>Student’s t-test</td>
<td>146</td>
<td>212</td>
<td>402</td>
<td>105</td>
<td>218</td>
<td>362</td>
<td>412</td>
<td>10</td>
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<tr>
<td>Cross-correlation</td>
<td>184</td>
<td>222</td>
<td>290</td>
<td>105</td>
<td>287</td>
<td>342</td>
<td>493</td>
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<tr>
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<td>203</td>
<td>277</td>
<td>111</td>
<td>242</td>
<td>387</td>
<td>433</td>
<td>9</td>
</tr>
</tbody>
</table>
FIG. 1. – Activation maps of one subject using the 8Hz on-off paradigm on 30 successive slices based on Z statistics (a), Student’s t-test (b), Spearman’s correlation (c), and cross-correlation (d). Red color indicates the area with neural activation.

FIG. 1. – Carte d’activation d’un sujet sain, paradigme 8 Hz on-off, 30 coupes successives, basée sur le score Z (a), le test de Student (b), le test de corrélation de Spearman (c) et la cross-correlation normalisée (d). Le rouge représente l’activation neurale.
somewhat arbitrary. Notice, that no consensus has been made about a common threshold (p-value), deciding whether a pixel show activation or not. The varying thresholds during the analyses employed in the current study with the 4 different statistical methods were therefore drawn from previously reported studies, which may add some limitations to our conclusions regarding the general comparison between the statistical methods. In return, one yields a detailed spatial distribution of the foci of activation that can be qualitatively compared. The pixel count metric is defined as the number of pixels in an image or a region-of-interest (ROI) having a small t score. If the statistical method used is the t-test, then this measure would be the sum of t scores above the chosen t score threshold. This measure could also be applied to other statistical measures such as correlation analysis. However, the sum of t scores is sensitive to changes in both spatial extent and intensity of activation [3].

The present study was conducted with a simple visual activation block paradigm, and four different statistical methods were considered appropriate to estimate statistical functional maps that represent neural activation in the brain. For more complex activation paradigms the brain responses may not be directly locked to the paradigm. Thus, in addition to classical statistical methods, other (more advanced) methods appropriate for spatial point distributions are applicable to fMRI data, including cluster analysis [2] and independent component analysis [17]. The average ROI signals have, for example, been used to analyze the relations between the intensity of the fMRI signal and behavioral variables in a paradigm [7]. Importantly, the use of integrated ROI signals has the advantage that no threshold enters the analysis but at the cost that a possibly spatially restricted change may vanish. Such a method also has the advantage of providing a continuous variable that is well suited for a variety of statistical analyses, including all classical techniques such as analysis of variance, regression, and tests of equality of means. An important criterion for the quality of dynamic time series is signal stability over time. It has been demonstrated that a SENSE-acceleration factor of 2 leads to a slight additional signal loss of a few percent only [9]. However, further scan time reduction by higher SENSE factors may lead to greatly increased spatially dependent noise related to the geometry of the coil array used, which will hinder appropriate statistical analysis. Neither echo planar nor anatomical images acquired with SENSE reduction factors have shown recognizable artifacts related to the applied parallel imaging technique within the brain [13], supporting our view that the statistical analysis is unaffected by this acquisition method.

CONCLUSION

The present study demonstrated that comparable parametric functional maps obtained during on-off block visual stimuli were found using different statistical approaches. Using Z statistics as the reference method, functional data showed similar findings when evaluated either by the Student’s t-test, Spearman’s correlation, or cross-correlation in no case showed very different findings, both when including the whole brain and the visual cortex, except when comparing the cross-correlation with z-clustering analyses in the whole brain. The neural activation of the brain was measured by simple pixel counting, and the number of activated pixels in the visual cortex was comparable for all 4 statistical methods, whereas analysis of the whole brain revealed a significant increased number of activated pixels when using the cross correlation and the Spearman’s correlation as compared with Z cluster statistics and Student’s t-test. This study therefore demonstrated that especially the Student’s t-test and Spearman’s correlation may lead to representative statistical information using fMRI data to analyze the activity of the visual cortex in response to a visual stimulus.

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REFERENCES

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