Journal of Cerebral Blood Flow and Metabolism
10:458–466 © 1990 Raven Press, Ltd., New York

The Relationship Between Global and Local Changes in PET Scans

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Summary: In order to localize cerebral cognitive or sensorimotor function, activation paradigms are being used in conjunction with PET measures of cerebral activity (e.g., rCBF). The changes in local cerebral activity have two components: a global, region independent change and a local or regional change. As the first step in localizing the regional effects of an activation, global variance must be removed by a normalization procedure. A simple normalization procedure is division of regional values by the whole brain mean. This requires the dependence of local activity on global activity to be one of simple proportionality. This is shown not to be the case. Furthermore, a systematic deviation from a proportional relationship across brain regions is demonstrated. Consequently, any normalization must be approached on a pixel-by-pixel basis by measuring the change in local activity and change in global activity. The changes associated with an activation can be partitioned into global and local effects according to two models: one assumes that the increase in local activity depends on global values and the other assumes independence. It is shown that the increase in activity due to a cognitive activation is independent of global activity. This independence of the (activation) condition effect and the confounding linear effect of global activity on observed local activity meet the requirements for an analysis of covariance, with the "nuisance" variable as global activity and the activation condition as the categorical independent variable. These conclusions are based on analysis of data from 24 scans: six conditions over four normal subjects using a verbal fluency paradigm. A technique is described based on ANCOVA and using statistical parametric mapping to localize foci in the brain that have been significantly perturbed by the cognitive tasks. This technique represents a fundamental and necessary departure from ROI-based approaches allowing the separation of global and local effects pixel by pixel, and provides an image of affected regions whose significance can be quantified. The specificity and sensitivity of the described method of change detection is assessed. **Key Words:** Positron emission tomography—Statistics—ANCOVA—Activation—Normalization.

There is an increasing use of specific activation paradigms in positon emission tomography (PET) studies of local cerebral function. Activation studies permit analysis of local changes in function brought about by cognitive, pharmacological, or sensorimotor challenge. One obstacle to the interpretation of regional data is the confounding effect of global changes.

Received September 18, 1989; revised December 13, 1989; accepted December 14, 1989.

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Abbreviations used: ANCOVA, analysis of covariance; PET, positron-emission tomography; rCBF, regional cerebral blood flow; ROI, region of interest; SSM, scaled subprofile model.

A number of methods have been proposed (Horwitz et al., 1984; Metter et al., 1984; Moeller et al., 1987) and criticized (Ford, 1986) that attempt to clarify the relations between regions or reveal consistent patterns of regional activity. The common problem for these and similar methods is to take account of global, region-independent, inter- and intrasubject variation. A simple approach to eliminate global effects is to divide regional values by the estimated average whole brain value as described for regional cerebral blood flow (rCBF) by Fox et al. (1988). This is probably a reasonable approximation but not necessarily the most sensitive solution and may be subject to errors.

Increases in local activity during activation may

be linked to global activity or they may be independent. Thus, an individual with intrinsically high global activity might show a larger, lower, or the same local increase during activation as a subject with low global activity. This can be seen in terms of the regression of local counts on whole brain counts. If the activation increase depends on global counts, then the regression for activation will be significantly steeper than for baseline conditions. Conversely, if the activation change is independent of global counts, then the regression lines will be parallel, with the activation condition consistently higher than the baseline condition. These two possibilities are illustrated in Fig. 1, for hypothetical data. The latter case meets the requirements for analysis of covariance (ANCOVA). This analysis assumes that a group effect (e.g., activation) and the effect of a confounding "nuisance" variable, the covariate (e.g., global flow), will combine to produce the variance in the observed dependent variable (e.g., rCBF). The global effect is removed by adjusting the observed values using an estimate of the global effect common to all activation conditions. The adjusted values are then compared to localize and quantify the change due to the activation(s).

In order to develop a method of data analysis that will be sensitive to small changes across groups or between activation conditions, we have examined first the relationship of local counts to whole brain counts. Second, the relative sensitivities of using differences in slope and differences in height of parallel regression lines, or intercept, were assessed using the same data.

The specificity of the technique described was estimated by applying ANCOVA to phantom images and calculating the probability of "false positives." Sensitivity was measured as the minimum change detectable at a confidence level that excluded false positives.

THEORY

Consider n scans obtained under essentially the same scanning conditions for different subjects. For m pixels and n subjects, the observed counts at any pixel i in subject j is given by C_{ij}. This is assumed to be dependent on two variables: a subject-independent relative value for that pixel (r_i) and a pixel-independent subject global count (G_j):

$$\text{Simple model: } C_{ij} = r_i \cdot G_j + e_{ij} \qquad (1)$$

Here r_i is the constant of proportionality for pixel i, which is invariant across subjects. e_{ij} is a normally distributed error term with a mean of 0. Equation (1) suggests that an increase in global counts is subtended by the same proportional increase in all regions and is a special case of the more general linear relationship:

$$C_{ij} = r_{0i} + r_{1i} \cdot G_j + e_{ij} \qquad (2)$$

with $r_{0i} = 0$. r_{0i} is the contribution to observed counts that is independent of global counts and r_{1i} reflects the dependency of local counts on global counts. The adequacy of Eq. (1) can be tested by regressing pixel counts on global counts and testing the null hypothesis that the intercept (r_{0i}) is zero using a t test.

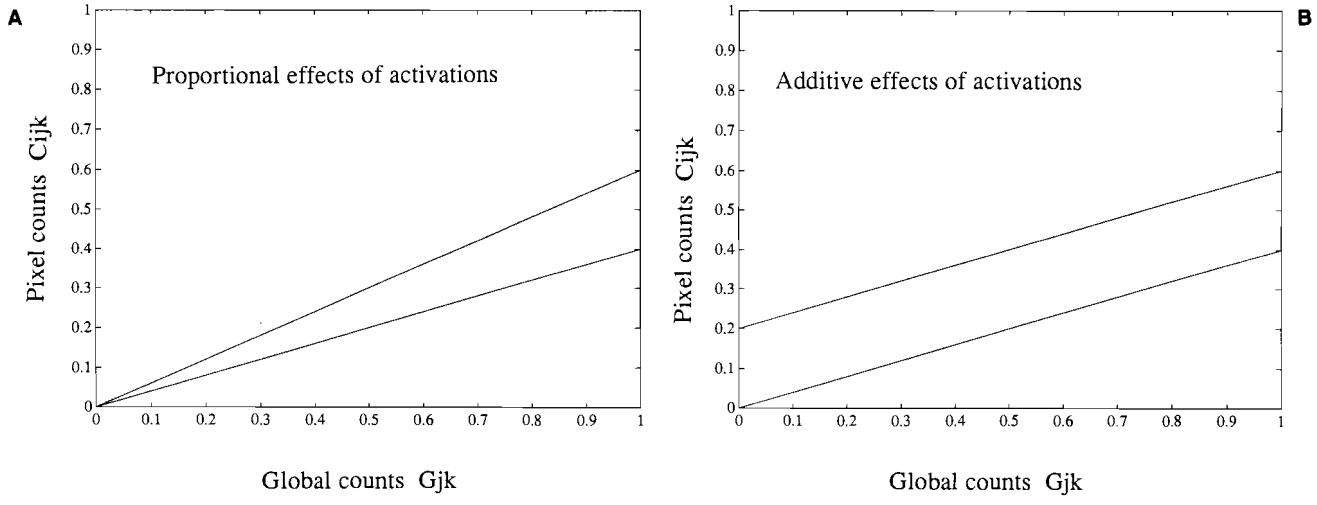

FIG. 1. Effects of activation according to the dependent and independent models in the text. The different lines correspond to different activation conditions. The regressions are of pixel values (C_{ijk}) on global counts (G_j). In Fig. 1A, the effect of the activation is to increase proportionally the pixel values above their baseline value, in this sense, the local or pixel change is dependent on global change. In Fig. 1B, the activation effect is additive and the same over the range of global counts (G_j); in other words, the local and global changes are independent.

The addition of the constant r_{0i} is important. For any one pixel, it is likely that the real relationship between that pixel's counts and whole brain counts will be nonlinear. Indeed, this must be the case if the above null hypothesis is rejected; otherwise, as global counts tend to zero, the pixel count will tend to r_{0i}. However, over a small range of global counts, a straight line will fit the data well. The addition of r_{0i} gives the regression an extra degree of freedom that allows for better modeling.

It is possible that the relative contributions of the arithmetic (r_{0i}) and proportional (r_{1i}) changes in local activity will not remain constant across brain regions. Another way of looking at this is to regard the constant of proportionality r_{1i} as the sensitivity of local change to global change. It is physiologically unlikely that the sensitivity to global activity in any one area is determined solely by its relative activity [as suggested by Eq. (1)]. It is more likely that this sensitivity relates to the neuronal projections to this area, the physiological range of activity permitted, and so on. The implications of this variability for change detection are clear. r_{0i} and r_{1i} must be estimated for each pixel by examining the behavior of that pixel's activity over a range of global activities. This is simply done by the regression analysis implicit in an ANCOVA. The physiological interpretation of r_{1i} as a sensitivity to global changes, or conversely stability in the face of global variability, can be demonstrated by examining how the intercept deviates from zero in a systematic way across brain regions. A large r_{0i} reflects a smaller regression slope than predicted by Eq. (1) and a relative insensitivity or resistance to global changes. A negative intercept would suggest increased relative sensitivity.

Now consider p scans under *different* conditions k. The relationship specified by Eq. (2) can accommodate a change (∂ik) in two ways. First, the increase (decrease) in region-dependent, subject-independent coefficient vector r_{xi} may be proportional or additive. This is modeled by an effect on r_{1i} and r_{0i}. If there is a component of change (∂ik_2) that reflects a change in slope (r_{1i}), then the overall effect on pixel counts depends on global counts. This is a dependent linear model:

$$\text{Dependent model: } C_{ijk} = (r_{0i} + \partial ik_1) + (r_{1i} + \partial ik_2)G_{jk} + e_{ijk} \quad (3)$$

If the effect of the activation is predominantly additive, then ∂ik_2 will be small and the dependent model reduces to

$$\text{Independent model: } C_{ijk} = (r_{0i} + \partial ik) + r_{1i} \cdot G_{jk} + e_{ijk} \quad (4)$$

An obvious way to test the relative validity of these models is to examine the regression slopes of C_{ijk} on G_{jk}. Clearly, for the dependent model, this slope should differ across condition k (Fig. 1A), and for the independent model, they should not, the main differences being in the intercepts (Fig. 1B).

The significance of differences between slopes, allowing for differences in intercepts, is given by the test for the homogeneity of regression coefficients (Wildt and Ahtola, 1978). This test statistic has the F distribution under the null hypothesis of no difference in slopes. Essentially, the difference in error variance between a model that permits different regression slopes for each condition and a model with a slope common to all is compared to the error variance using the F ratio. A statistical parametric map of this F value for each pixel i can be displayed as an image. This image would highlight any area "perturbed" according to a dependent model.

The significance of difference in intercepts of the regression lines is assessed for each pixel using the omnibus F ratio of variance between adjusted condition means and the adjusted error variance. This is treating the independent model as a one-way layout covariance model with global counts as confounding covariate. The equivalent statistical map would be sensitive to changes independent of global activity.

For a full description of the calculations, see Wildt and Ahtola (1978). In effect, ANCOVA removes variance due to differences in global flow, leaving only that variance due to the categorical independent variable, in this case the activation or condition. The analysis is performed for each pixel independently. The F ratio of adjusted treatment over error variance provides an omnibus test for perturbation at any pixel i. This ratio can be thought of as the distance between regression lines divided by the average scatter about these lines for each condition.

The significance of any change from a single condition to any other or between groups of conditions involves a comparison of adjusted condition means using the t statistic and a Bonferroni correction in the case of nonorthogonal comparisons.

Comparison of dependent and independent models

For a large number of pixels analyzed in this way, the number of pixels reaching a certain level of significance can be measured. If the dependent relationship is the best description, the number of suprathreshold pixels from the test of inhomogeneity of regression coefficients should be greater than expected by chance and the number from the omnibus

F about that expected by chance. If the independent relationship is valid, the opposite will be found. The probability of observing a given number of suprathreshold pixels by chance is simply calculated. This probability is also the significance of the statistical map as a whole. If the threshold for "significance" is set high enough and a large enough number of pixels are compared, then the probability that x pixels, or more, would be significant by chance can be calculated from the Poisson distribution. The Poisson distribution can be obtained by a limiting process applied to the binomial distribution. It can be shown (Collings, 1977) that

$$P(X = x) = \frac{e^{-q}(q)^x}{x!} \quad (5)$$

where

$$q = m \cdot t \quad (6)$$

q is the parameter of the Poisson distribution and m and t define the binomial distribution $b(m,t)$ approximated. In this case, m is the number of pixels included in the analysis and t is the probability threshold chosen. From Eqs. (5) and (6), the probability that the number of observed pixels with values corresponding to a significance level of t or above have occurred by chance can be calculated. This probability is the significance of the statistical map or landscape as a whole.

This form of omnibus testing of the image slice has no localizing power but represents a nonarbitrary test for outliers in the F distribution. It is nonarbitrary because the pixels subtending the "improbability" of chance occurrence are explicitly identified by the threshold chosen (t).

METHODS

Subjects and activation paradigm

The subjects were four right-handed male volunteers (age of 26–45 years) who had no present or past history of neurological or psychiatric symptoms. Each subject underwent six consecutive studies whilst performing different verbal fluency tasks. The details of these tasks and neurophysiological findings will be reported in a separate paper. In brief, the first and last studies were under standardized relaxation conditions and constitute the baselines. The remaining four conditions involved self-paced verbal fluency tasks with verbalization and in some conditions listening to words presented aurally.

Data acquisition

Scanning was performed using a PET scanner (CTI Knoxville model 931-08/12 CTI PET Systems, Knoxville, TN) whose physical characteristics have been described (Spinks et al., 1988). For the present studies, scans were reconstructed using a Hanning filter with a cutoff frequency of 0.5, giving a transaxial resolution of 8.5 mm full width at half-maximum. The reconstructed images contained 128 × 128 pixels, each having a size of 2.05 × 2.05 mm.

Subjects inhaled $C^{15}O_2$ at a concentration of 6 MBq/ml and a flow rate of 500 ml/min through a standard oxygen face mask for a period of 2 min. Dynamic PET scans were collected for a period of 3.5 min starting 0.5 min prior to $C^{15}O_2$ delivery, according to a protocol described elsewhere (Lammertsma et al., 1989) used for measuring rCBF. For the present study, integrated counts per pixel for the 2 min buildup phase of activity during $C^{15}O_2$ inhalation were used.

Preliminary image analysis

Image analysis was performed on a Sun workstation (3/60) (Sun Microsystems Europe, Inc., Surrey, UK) using an interactive image analysis software package (ANALYZE, Biodynamic Research Unit, Mayo Clinic, Rochester, MN, U.S.A.). Calculations and image matrix manipulations were performed in PRO MATLAB (Mathworks Inc., Sherborn, MA).

The 15 original scan slices (6.75 mm interplane distance) were interpolated, using bilinear interpolation to 26 planes into a standard stereotactic space. The intercommissural line was identified, according to a previously described method, directly from the primary PET image (Friston et al., 1989). When stereotactically normalized, one pixel in the transformed image represents 2 mm in the x and y dimensions according to the atlas of Talairach et al. (1988). The brain image falls within the central 65 × 87 submatrix of the 128 × 128 image. The slices have an effective interplanar distance of 4 mm. Significant areas within the statistical parametric maps were displayed with the proportional "grid" and brain outline used by the atlas (Talairach et al., 1988). Each slice corresponds to a horizontal section in the atlas. In order to accommodate variance in position due to head movement, nonlinear differences in the shape of the subjects' brains, and errors in anatomical localization, each image was smoothed using a low-pass filter of nine pixels square. This markedly increases the signal-to-noise ratio.

Statistical analysis

The global counts (G_{jk}) were determined by summing counts in all pixels between −8 and +32 mm relative to the intercommissural (AC–PC) plane. C_{ijk} were the counts for pixel i (1–128 × 128 × 26) in subject j (1–4) for condition k (1–6).

Test of simple proportional model

Using only baseline scans (conditions 1 and 6) in the four subjects, the eight pixel values (C_{ij}) for each pixel were regressed on the corresponding global count G_j. The intercept was calculated for every pixel between −8 and +32 mm relative to the intercommissural line. The t statistic was used to test the null hypothesis that the mean intercept across pixels was zero. Note that the adequacy of Eq. (1) is being tested both between subjects and over time within subjects.

To determine if any systematic deviation from the null hypothesis, over brain regions, existed, the correlation between mean activity and intercept was derived over all pixels. To illustrate any systematic differences, a descriptive map was generated whose pixel values were the observed intercept.

Test of dependent and independent models

Using all the 24 scans (conditions 1–6), for each pixel two F values were calculated as a test for inhomogeneity

of regression coefficients and for treatment effect on adjusted means. These F values were calculated according to a randomized block design ANCOVA. The systematic intersubject (block) variance is removed by partitioning the error variance into true error variance and this block effect. The resulting statistical parametric maps (SPMs) were generated and thresholded at $p = 0.001$. The significant areas were plotted on the proportional grid and brain outline used by the Talairach atlas (1988). The first SPM represents the significance of change in the regression coefficients calculated for each condition separately. This corresponds to a test of the dependent model. The second SPM shows areas of change according to absolute changes once the variance due to global differences has been removed—independent changes.

To assess the significance of the SPMs generated above, a threshold of $p = 0.001$ was chosen and the number of suprathreshold pixels in each SPM counted. Given that the SPM represents 65×87 repeated pixel measurements, the parameters in Eqs. (5) and (6) are defined. Only pixels whose mean activity was one-third of the maximum were included in the analysis. The overall significance of the SPMs is given by the probability that the number of significant pixels or more would have been seen by chance.

Specificity of ANCOVA

ANCOVA with global counts as confounding covariate was applied to 24 uniform phantom images used in calibration. The layout was identical to that used in the analysis of real data above, with four images for each of the six conditions. The design in this case was completely randomized since there was no block effect. These phantom images were circular and had total counts equivalent to the brain images. The phantoms were smoothed to the same extent as the real images. The omnibus F value for each pixel was calculated according to the one-way layout of covariance model as described. This whole procedure was repeated 26 times on independent phantom images to simulate the 26 planes of brain images. The cumulative distribution of the F value was compared to that predicted in the absence of any change. This was a further text of statistical validity of the procedure. The probability of obtaining the observed number of suprathreshold pixels (at $p = 0.001$) by chance was calculated.

Sensitivity of ANCOVA

For the complete analysis of the real data above, the greatest percentage increase in activity from one condition to another, a change score, was recorded for all pixels with an F value at $p < 0.001$. These change scores were derived from the adjusted group or condition means. The frequency distribution of the change scores detected was derived for all planes.

RESULTS

Proportionality

The mean intercept (r_{0i}) was 0.708% of mean pixel activity. This is significantly different from zero ($t = 16.31$, $p < 0.00001$). The null hypothesis that the intercept is zero can be rejected.

Furthermore, there was a significant negative correlation between the mean activity for each pixel and its corresponding intercept ($r = -0.3133$, $p < 0.001$). This suggests that brain regions with higher cerebral activity are more sensitive to global changes than would have been predicted by the simple proportional relationship.

This systematic deviation from a simple proportional relationship in specific brain areas is illustrated in Fig. 2. This slice is a horizontal section 8 mm above the AC–PC line and corresponds to Fig. 117 in the atlas of Talairach et al. (1988). The areas shown have intercepts two standard deviations below the mean and include the striate and extrastriate cortex, and the posterior and anterior cingulate cortex. These areas are generously connected to large areas of cortex and themselves have relatively high activity. Their increased sensitivity to global changes may, in part, be due to these characteristics.

In light of these findings, a simple proportional model is rejected in favour of the more general relationship described by Eq. (2), which forms the basis of the dependent and independent models.

Dependence vs. independence

The SPM based on an ANCOVA with global counts as covariate and condition as the independent categorical variable was found to be more sen-

FIG. 2. Brain areas whose intercepts observed on regressing pixel counts on global counts fall two standard deviations below the mean for this slice. The low intercept reflects a relatively higher sensitivity to global changes than would have been expected by a proportional model with an intercept of zero. The slice is 8 mm above the AC–PC (intercommissural) line and corresponds to Fig. 117 in the atlas of Talairach et al. (1988). The grid superposed on the image corresponds to that in the atlas.

sitive to change than the SPM based on dependence. Figure 3 shows the same slice as Fig. 2. Figure 3A represents a small area in the posterior temporal cortex (Brodmann's areas 21/37) that has different slopes. Compare this with the two larger areas of pertubation in Fig. 3B representing a condition effect independent of global activity. These areas are in the auditory association cortex (left Brodmann's area 42) and left anterior insular cortex. The former area was predicted since some conditions involved listening and others did not.

As a test of significance of these areas, the actual and expected number of pixels above a threshold were compared for both SPMs. The number of pixels greater than $p = 0.001$ in Fig. 3A is two. For Fig. 3B, the number is 17. For this threshold and SPM size, the probability of obtaining at least two pixels by chance is 0.954 and in fact the number of pixels observed is less than that expected. This means the SPM based on difference in slopes is not significant and the area of activation has most likely arisen by chance. However, for the ANCOVA SPM, the probability of getting 17 pixels by chance is low ($p < 0.009$) and the significance of this SPM is correspondingly high. Indeed, over the whole brain, the ratio of pixels with different intercepts to pixels with different slopes was 3.47. For different slopes, 36 pixels were observed at $p = 0.001$. This is one-half the number expected. For significantly different intercepts, 125 pixels were observed, a highly unlikely chance occurrence ($p < 0.00001$).

In the ANCOVA analysis, pixels with different regression slopes are excluded from further analysis.

These findings are consistent with an independence of local and global changes.

Specificity

Figure 4 shows the measured F distribution from the phantom ANCOVA analyses and that predicted theoretically. The agreement is evident. Of note is that for the higher values of F, there is no value for which the observed number of pixels exceeded that expected. Consequently, using the criteria described above to assess the significance of the plane as a whole, few false positives should arise.

Using a threshold of $p = 0.001$, an average of 4.38 suprathreshold pixels was observed per plane. This is less than expected (5.6) and the probability of this number or more occurring by chance is 0.994.

Sensitivity

For this cognitive paradigm, the magnitude of differences detected using an ANCOVA as described has a median in the range of 12–15%. The smallest changes detected in this data set are on the order of 6.5%. This is using a confidence level of $p = 0.001$, which is conservative but is unlikely to give false positives (Fig. 5).

DISCUSSION

Analysis of the relationship between pixel values and global counts suggests the following: (a) The

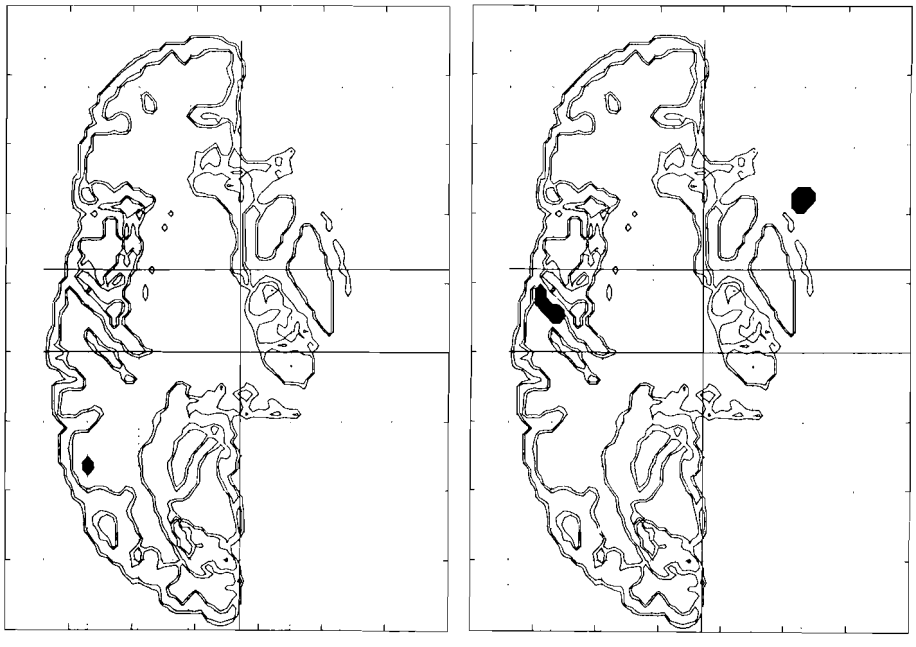

FIG. 3. The same slice as in Fig. 2. **(Left):** Statistical parametric map (SPM) according to the dependence model where the value at pixel i is given by an F value reflecting inhomogeneity of the regression coefficients when calculated separately for each condition. This F statistic is a test of significant difference in the slopes of the regressions of pixel counts on total counts for each condition. See the main text for a full discussion. The areas shown are those with an F value at $p < 0.001$. **(Right):** SPM according to the independence model, where the pixel values are given by the omnibus F value for a treatment effect. This F statistic is a test of significant difference between the heights of the regression lines, or intercept, using an estimate of the regression coefficient that is common to conditions. See the main text for descriptions of the regions highlighted.

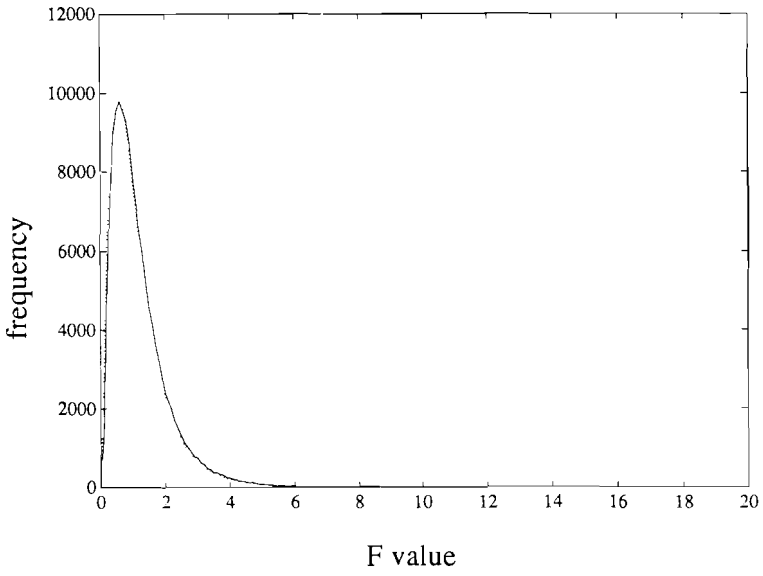

FIG. 4. The observed and predicted distribution of *F* values for an ANCOVA on phantom images. These phantoms are uniform and therefore there can be no regional change once global variance has been removed. The degrees of freedom of this distribution are 5 and 17.

global, region-independent effects cannot be accounted for by a simple scalar effect on regional values. In particular, a simple proportional relationship between regional and whole brain activity cannot be assumed in that brain regions differ in their sensitivity to global changes. This can be taken as justification for a more general (but still linear) formulation [Eq. (2)] that in a sense incorporates both proportional and independent effects. (b) The magnitude of change due to the activation does not depend on global counts, and a one-way layout ANCOVA is an appropriate approach to partitioning out the effects of global differences (Fig. 6). (c) Because the dependence of local on global activity may vary across the brain, a pixel-by-pixel analysis is required for valid normalization. (d) Although conservative, a confidence threshold of p = 0.001 makes the analysis specific without grossly affecting sensitivity.

There are a number of alternative approaches to identifying change in activation studies using PET. Some approaches using a region of interest (ROI)-based technique (Roland et al., 1987) do not attempt to differentiate between relative and global effects. For large ROIs, any change relates to a difficult to define concept—a regional global effect. This reflects the size of region examined and the questions

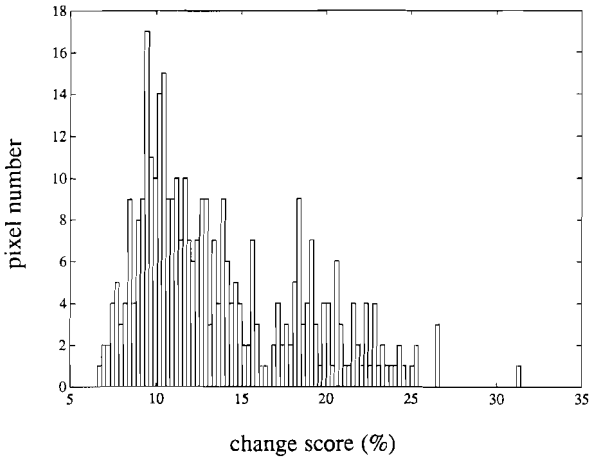

FIG. 5. Frequency distribution of the greatest percent change at pixel locations that were identified using ANCOVA with a significance level of p = 0.001. This distribution results from an analysis of the whole brain across the six cognitive states described in the text. The minimum percent change to which this analytic technique appears to be sensitive is 6.58%.

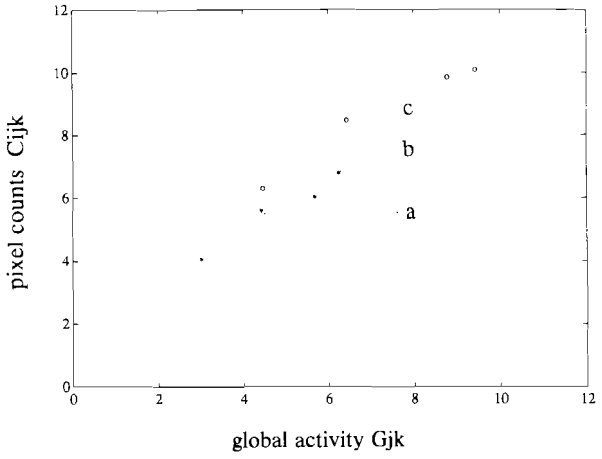

FIG. 6. Regression of pixel counts on global counts illustrating the layout of the ANCOVA. The asterisks (*) are the four subjects at baseline and the circles (○) the activated brain state (orthographic word generation). The distance *ac* represents the total increase in the average pixel activity for the four subjects. This has two components: a component due to the increase in global activity (*ab*) and the true local effect of the activation (*bc*). The regression coefficient is calculated from all 24 data points after correcting for differences in group means. The points plotted are from a pixel in the left Brodmann's area 46.

asked of the data. Obviously, if the activation produces a significant change in a large ROI, by virtue of its size this change would contribute to a global change. The assumption of independence cannot hold in this situation and the arguments developed in this paper cannot be applied. This illustrates a fundamental departure of ANCOVA with statistical parametric mapping from conventional image analysis since it eschews ROI-based techniques. The concept of relative focal change depends on a definition of a global reference against which the relative change is measured. Obviously, since global activity is merely the sum of all focal activities, true independence is never the case. However, the best estimate of a reference that is invariant despite small local relative changes remains the global activity. The smaller the effect of local measure on global estimate, the better this estimate will be. This is a further argument for analyzing, by measuring one pixel at a time. In short, pixel-by-pixel analysis allows for assumptions of independence of local change from the whole image.

The normalization adopted by an existing method of change detection in PET images (Fox et al., 1988) involves division by whole brain mean. Although this approach has little empirical support from the findings of this study, it may still be a reasonable approximation for large changes. It must be pointed out that although the Scaled Subprofile Model (SSM) of Moeller et al. (1987) uses a model similar to the dependence model, the global scaling factor of that technique is quantitatively determined by a model-driven formulation; hence, the present findings have little relevance to the SSM. Furthermore, analyses of covariance that seek to determine the covariance of pixel values in different parts of the brain have a different goal from the simple detection of change. The model discussed by Ford (1986) bears similarities with the preferred model in this paper in that the "patient effect" is additive, but the dangers he highlights are not directly relevant to "change detection."

The nonsphericity or high covariance of nearby pixels has no effect on the ANCOVA as described since a pixel-by-pixel analysis requires the data to come from different scans. The distribution and hence interpretation of the F value is unaffected by smoothing, as evidenced by the results presented in Fig. 4. There is an effect on the distribution of F values in space, however. Although the same number of specified F values will be observed, as would have been in the absence of smoothing, like-valued F values will tend to cluster together. This should be remembered when interpreting the SPMs. For example, if a threshold of $p = 0.01$ is used for the analysis of 1,000 pixels, 10 pixels would be expected by chance. However, if pixels cluster in groups of 10, only one false positive is expected. This is obviously irrelevant in an hypothesis-led or confirmatory study, but if the described approach is used in an exploratory fashion, a Bonferroni correction will be required. This correction will depend on the degree of smoothing and on an estimate of the "effective" number of independent samples. In the above example, the effective number of repeated measures is only 100. The estimation of the number of effective independent samples in well-behaved smooth images will be discussed in a separate paper.

A simple method of quantifying the significance of the final SPMs is described and calculates the probability that the SPM could have arisen by chance. Although this approach is conceptually and computationally simple, it does not address the problem of interpretation. In assuming that the SPM "landscape" as a whole is highly significant, there can be no assumptions about which "features" are significant, e.g., whether one focus of activation is significant or merely a collection of high-valued pixels that one would expect by chance. There are a number of approaches to this problem. First, there are qualitative aspects of foci of activation, such as size and shape, that would suggest minimal probability of chance occurrence. Second, and more powerfully, the covariance structure of the images should guide interpretation of omnibus SPM. In brief, once the significance of the whole SPM has been established, interpretation should not be limited by post hoc statistical considerations alone but should call on different approaches and knowledge about the underlying neurobiology.

The design of the ANCOVA will obviously depend on experimental design. One important choice will depend on the difference between intersubject and intrasubject variability. If all activation conditions are performed in every subject, then the most appropriate analysis would be a randomized block design. In this layout, the systematic intersubject differences are removed from the error variance and the sensitivity to change increased. If, however, activation conditions have been performed in different subjects, a completely randomized design is appropriate.

The conclusions reached above must be qualified by the fact that although 24 studies were performed, only four subjects were used. The assumption that activation increases are not dependent on global activity can be tested empirically in longitudinal stud-

ies of single subjects using time effects to introduce global variability.

A thorough assessment of this technique's sensitivity cannot be performed with the uniform phantoms used (although specificity can be judged). Simulation of regional differences in the context of global variance is required. Both of these studies are currently being undertaken in this unit.

CONCLUSION

The use of ANCOVA in removing global subject effects and determining the significance of perturbation by specific activations has been described and compared to other models. The results suggest that the covariance model, with global counts as the confounding (or "nuisance") covariate and condition (or pathology) as the categorical variable, is a suitable and sensitive approach to detecting change.

Acknowledgment: K.J.F. is funded by the Wellcome Trust. We thank our colleagues at the MRC Cyclotron Unit for making the studies possible.

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