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Is Multivariate Analysis of PET Data More Revealing Than the Univariate Approach? Evidence from a Study of Episodic Memory Retrieval

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In a functional imaging study of cued paired associate retrieval, in which the strength of association between pair members was systematically varied, we predicted increased right frontal activity as a function of weakening semantic linkage. An initial univariate analysis found the opposite effect, with greater right frontal activity during recall of strongly linked paired associates. This unexpected result led us to perform a multivariate analysis of covariance (MANCOVA), an approach which proved more informative. This analysis showed that the most significant source of task-related variance was accounted for by a nonlinear relationship not predicted by the prior hypothesis and not revealed by the standard univariate approach. This application of the MANCOVA supports the assertion that multivariate analysis can provide an important adjunct to univariate approaches like statistical parametric mapping (SPM). New perspectives engendered by the MANCOVA still allow for statistical inference but are not constrained by explicit hypotheses about specific task-dependent effects.

INTRODUCTION

Functional imaging studies of memory have implicated the right frontal lobe in episodic memory retrieval (Shallice *et al.*, 1994; Tulving *et al.*, 1994a; Squire *et al.*, 1992), and hypotheses have been put forward concerning the role which it might play. It has been suggested that this area may be involved in monitoring and verification processes necessary for successful retrieval (Shallice *et al.*, 1994). These terms refer to processes to which a candidate response must be subjected, i.e., response assessment in relation to the demands or context of the task (monitoring) and confirmation of the appropriateness of a response (verification) will precede its initiation. On the basis of this hypothesis, we speculated that random paired associates would require more monitoring and verification of candidate responses than would semantically related items since, when items are related, the stimulus constrains the semantic field in which the response is likely to lie. Put simply, randomly linked pairs need stricter checking.

In this study of cued paired associate retrieval, we parametrically varied the strength of semantic association between paired associates. Association varied from strong to weak over five levels (strength 5 through to strength 1). A sixth condition (strength 0) employed pairs which were unrelated. While the cognitive processes engaged by this change in semantic association are speculative, it is true that most distantly related pairs (i.e., semantically unrelated pairs, e.g., cue—"*goose*" . . . response—"*swamp*") are less easily recalled than closely linked pairs (e.g., cue—"*dog*" . . . response—"*cat*") and semantically distant responses require a greater degree of examination and assessment ("monitoring/verification") before being accepted as appropriate. Our expectation was that increasing semantic association would reduce the extent to which monitoring and verification processes were employed.

The neural correlates of this reduction are, again, speculative, but there are grounds for suggesting a frontal component. For example, patients with frontal damage can be grossly impaired in the retrieval of unrelated paired associates unless the pairs are presented as part of a sentence which engenders a link between them (Signoret and Lhermitte, 1976). Although this finding was interpreted as a frontal damage-related failure of organization at encoding, it is equally plausible that the provision of this semantic link at encoding was an aid to the monitoring/verification processes at the point of retrieval. Evidence for this comes from a more recent study in which the provision of semantic "structure" facilitated recall in frontally damaged patients only when the "structure" was supplied at retrieval as well as encoding but not at encoding alone (Incissa Della Rochetta and Milner, 1993). Another patient, RW (Derouesné *et al.*, 1985;

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Shallice, 1988), was unable to perform paired associate recall when stimuli elicited an irrelevant association. A failure to disregard such irrelevant associations may be viewed as a dysfunction of the monitoring/verification process and appears to be a core deficit underlying confabulation, the apparent recall of past events which, while held with conviction, is grossly unreliable (Shallice, 1988). The predominant pathology associated with confabulation lies in the medial frontal lobes (Kapur and Coughlan, 1980), although, more recently, it has been suggested that it is associated with right-sided lesions (Stuss *et al.*, 1993). Thus there are good reasons for making our anatomical hypothesis quite specific, especially as previous functional imaging studies have related episodic retrieval to right prefrontal activity.

The main purpose of this paper is to show that, by avoiding the constrained hypotheses applied in the standard univariate analysis (i.e., hypotheses which are implicit in the specification of the contrasts), one can test the more general hypothesis that there are differences between tasks about which one can make a statistical inference. Having made this inference, the nature of these differences can be characterized post hoc. This unconstrained characterization revealed effects in our data which had not been predicted and which could have been overlooked using the standard univariate approaches such as statistical parametric mapping. This new approach can be thought of as combining the statistical inference of standard univariate techniques with the exploratory usefulness of data-led approaches such as principal components analysis (i.e., eigenimage analysis).

MATERIALS AND METHODS

Subjects

Six right-handed male subjects (age range 22–30 years) took part in the study. All subjects were healthy and free from any history of neurological or psychiatric illness. The study was approved by the local hospital ethics committee and the Administration of Radioactive Substances Advisory Committee (United Kingdom) (ARSAC).

PET Scanning

Each subject underwent 12 PET estimations of brain activity over a 2-h period. Scans were obtained using a CTI Model 953B PET Scanner (CTI, Knoxville, TN) with collimating septa retracted. Volunteers received a 20-s intravenous bolus of $H_2{}^{15}O$ at a concentration of 55 MBq ml^{-1} and a flow rate of 10 ml min^{-1} through a forearm cannula.

Psychological Tasks

Subjects were scanned during cued paired associate retrieval. Each word pair list, consisting of 12 pairs, was presented 5 min prior to the PET scan. During each PET scan, subjects were cued with the first member of each pair and required to respond with the appropriate associate.

Pair associations were rated on scale with 5 indicating a close relationship, 1 indicating a distant relationship, and 0 indicating no relationship (Keppel and Strand, 1970).

Each subject received six lists of imageable and six lists of nonimageable paired associates (one list was presented with each PET scan). This part of the experimental design is described elsewhere (Fletcher *et al.*, 1995).

We expected that the weakly linked pairs would be more difficult to recall, leading to differential performance as a potentially confounding factor. On the basis of a pilot study (using a different but comparable group of subjects) we varied the number of prescan presentations to maintain constant levels of performance across the 12 scans. The overall study design is summarized in Fig. 1.

Data Analysis

The data were analyzed with statistical parametric mapping (using SPM95 software from the Wellcome Department of Cognitive Neurology, London, UK) implemented in Matlab (Mathworks Inc., Sherborn MA, U.S.A.). Statistical parametric mapping combines the general linear model (to create a statistical parametric map or SPM) and the theory of Gaussian fields to make statistical inferences about regional effects (Friston *et al.*, 1991, 1994; Worsley *et al.*, 1992). In this application we used only uncorrected *P* values because of our regionally specific hypotheses.

The scans from each subject were realigned using the first as reference. Following realignment, all images were transformed into a standard space (Talairach and Tournoux, 1988). This normalizing spatial transformation matches each scan to a reference template image that conforms to the standard space (Friston *et al.*, 1996a). As a final preprocessing step, the images were smoothed using an isotropic (16-mm FWHM) Gaussian kernel.

Univariate Analysis

The condition, subject, and covariate effects were estimated according to the general linear model at each voxel (Friston *et al.*, 1995). To test hypotheses about regionally specific condition effects, the estimates were compared using linear compounds or contrasts. The

Strength of Semantic Link	IMAGEABLE PAIRS	NON-IMAGEABLE PAIRS
5	1 pre-scan presentation	1 pre-scan presentation
4	1 pre-scan presentation	2 pre-scan presentations
3	2 pre-scan presentations	3 pre-scan presentations
2	2 pre-scan presentations	3 pre-scan presentations
1	3 pre-scan presetations	4 pre-scan presentations
0	4 pre-scan presentations	8 pre-scan presentations

FIG. 1. Diagrammatic representation of the study design. Twelve lists were presented to each subject in counterbalanced order. Each list was designated either imageable or nonimageable and varied along a scale of 5 to 0 indicating strength of association between pair members. To equalize performance across the 12 scans, the number of prescan presentation was varied as shown.

resulting set of voxel values for each contrast constitute a statistical parametric map of the t statistic, SPMt. The SPMt were transformed to the unit normal distribution (SPMZ) and thresholded at 3.09 (or $P = 0.001$ uncorrected for multiple comparisons).

We evaluated rCBF responses to variation in strength of semantic association. This analysis was performed by weighting the conditions (from semantic distances "6" to "0") -5, -3, -1, 1, 3, 5 (to show increases in activity with increasing semantic distance) and 5, 3, 1, -1, -3, -5 (to show decreases). This analysis showed a relative *decrease* in frontal lobe activity as the semantic distance increased.

Multivariate Analysis

Following this counterintuitive finding, we performed a multivariate analysis of the data. A full account of this method is described by Friston *et al.* (1996b). It involves assessment of the significance of condition-dependent effects, having transformed the data using their principle components analysis. The 12 scans are simply treated as different conditions without constraining the analysis by specifying anything further about the tasks. The (transformed) image is treated as a single multivariate observation, and changes in brain activity are assessed with regard to their significance using Wilk's lambda. These changes are then characterized in terms of a set of canonical images using canonical variates analysis. Canonical images can be thought of as eigenimages or principal components of the condition effects, after discounting interactions due to error. For each canonical image, there is a corresponding time series that reflects the extent to which each image is expressed over the conditions. This time series is called a canonical vector or variate.

In summary, the approach gives:

1. A P value using Wilk's lambda.
2. A small number of canonical images.
3. Their associated canonical vectors.

RESULTS

MANCOVA and Wilk's Lambda

The design matrix had 12 columns representing the 12 conditions. These are represented to the left of the design matrix (see Fig. 2a). We designated subject effects and global activity as uninteresting confounds. These are represented to the right of the condition effects. The partition of the design matrix had 5 columns for each subject (subject effects were constrained to zero, eschewing the need to incorporate the sixth subject effect explicitly). The final column contains global activities. Condition-specific effects were very significant ($P < 0.00001$). In other words, the probability of there being no difference between the 12 conditions was <0.00001.

Canonical Variates Analysis

The condition effects were almost entirely accounted for by two canonical images, mostly by the first (see Fig. 2b). The first canonical image and its expression under each condition are shown in Fig. 3a. The top left panel shows the positive components of this image which include the right prefrontal cortex.

The canonical variate expressed in terms of mean condition effects is seen in Fig. 3b. This indicates that frontal flow is positively associated with increased strength of semantic association across the first five conditions (for both the imageable and the nonimageable material) only. The relationship is completely reversed for the totally unassociated pairs (strength = 0).

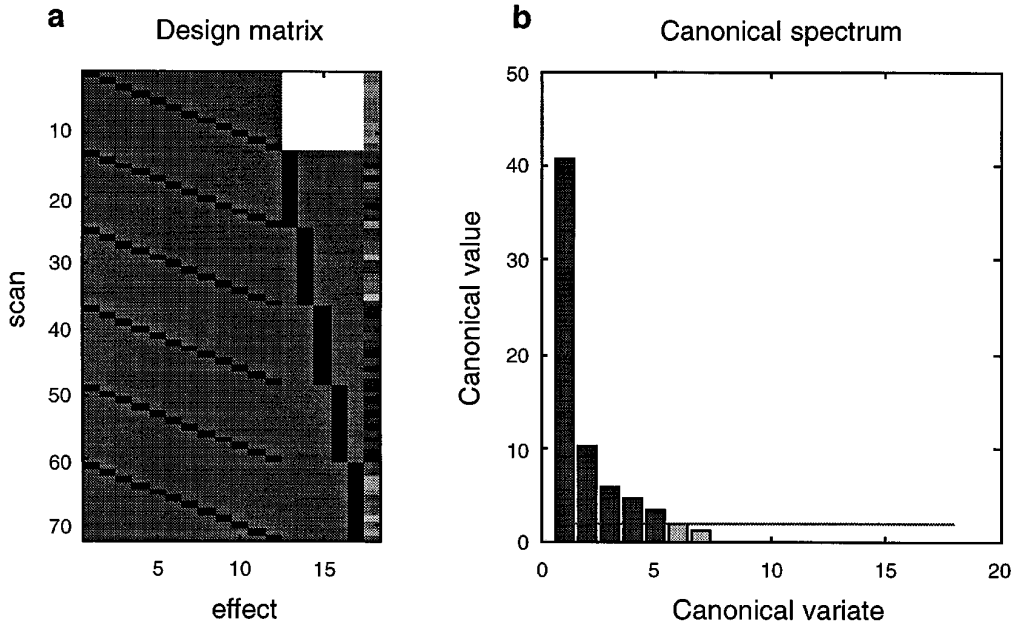

FIG. 2. (a) Design matrix. The 12 condition effects are shown to the left. The 6 confounds are shown to the right. The matrix is displayed in image format with each column scaled to its maximum. This matrix is described more fully in the text. (b) The spectrum of canonical values following a canonical variates analysis of the sums of squares and products matrices due to condition and error terms. The dark bars represent canonical values that exceed $F_{0.05(h,r)}$. $\chi^2 = 305.93$. The threshold of $P = 0.05$ is 231.83. $P = 0.00001$.

Further SPM Analysis Informed by the Multivariate Approach

The above multivariate analysis indicated a qualitative difference between the semantically linked pair retrieval (strengths 5 to 1) and the unlinked retrieval (strength 0). We therefore returned to the standard SPM analysis and reanalyzed the data in two ways. First, we excluded the unlinked pair condition and simply analyzed changes in brain activity with the changing semantic relationship across the linked conditions. This showed a strong frontal decrease with decreasing strength of semantic relationship between the pairs. Second, we performed a direct comparison between the unlinked pair conditions and the linked pair conditions. This showed that, whereas there is a frontal decrease in activity as the semantic relationship is lessened, there is a strong increase when subjects recall unlinked pairs (see Fig. 4).

DISCUSSION

Our novel multivariate analysis has shaped our interpretation of the data in a number of ways. First, the effect of the conditions (different semantic strengths) incorporated into the study design is a significant one. Second, it indicates that the initial prediction, that frontal flow would increase in a linear fashion as associated pair members became less strongly related, was wrong. Third, and most informatively, it characterizes the pattern of frontal flow as a decrease across semantic associations 5 to 1 with a reversal of this pattern when the semantic link is broken (strength = "0"). This indicates, in effect, that the condition of retrieving semantically unlinked items is qualitatively different from those in which items were linked. This phenomenon might be missed using the standard statistical inference in univariate analyses of the data. A revised univariate analysis (in which the unlinked pairs were not seen as part of the parametric variation) provides a better interpretation of the results in terms of the frontal contribution to episodic memory retrieval.

Our initial, specious interpretation, which the MANCOVA demonstrated to be inadequate, was that the results reflect a practice effect superimposed onto the effects of semantic manipulation. As Fig. 1 shows, paired associates which were more strongly linked required fewer numbers of prescan presentations to equalize performance during scanning. Thus, the decreasing degree of item novelty from semantic linkages "5" to "0" (introduced to ensure equal performance across all scans) may account for the frontal decreases. This would fit in with previous studies on the effects of novelty on cerebral activity (Raichle *et al.*, 1994; Tulving *et al.*, 1994b). However, at best, it is a partial explanation since, as Figs. 3b and 4 show, right frontal activity decreases only from linkages "5" to "1," for both imageable and nonimageable recall. There is an increase for the randomly associated pairs. Since these pairs were the least novel, a practice-related decrease

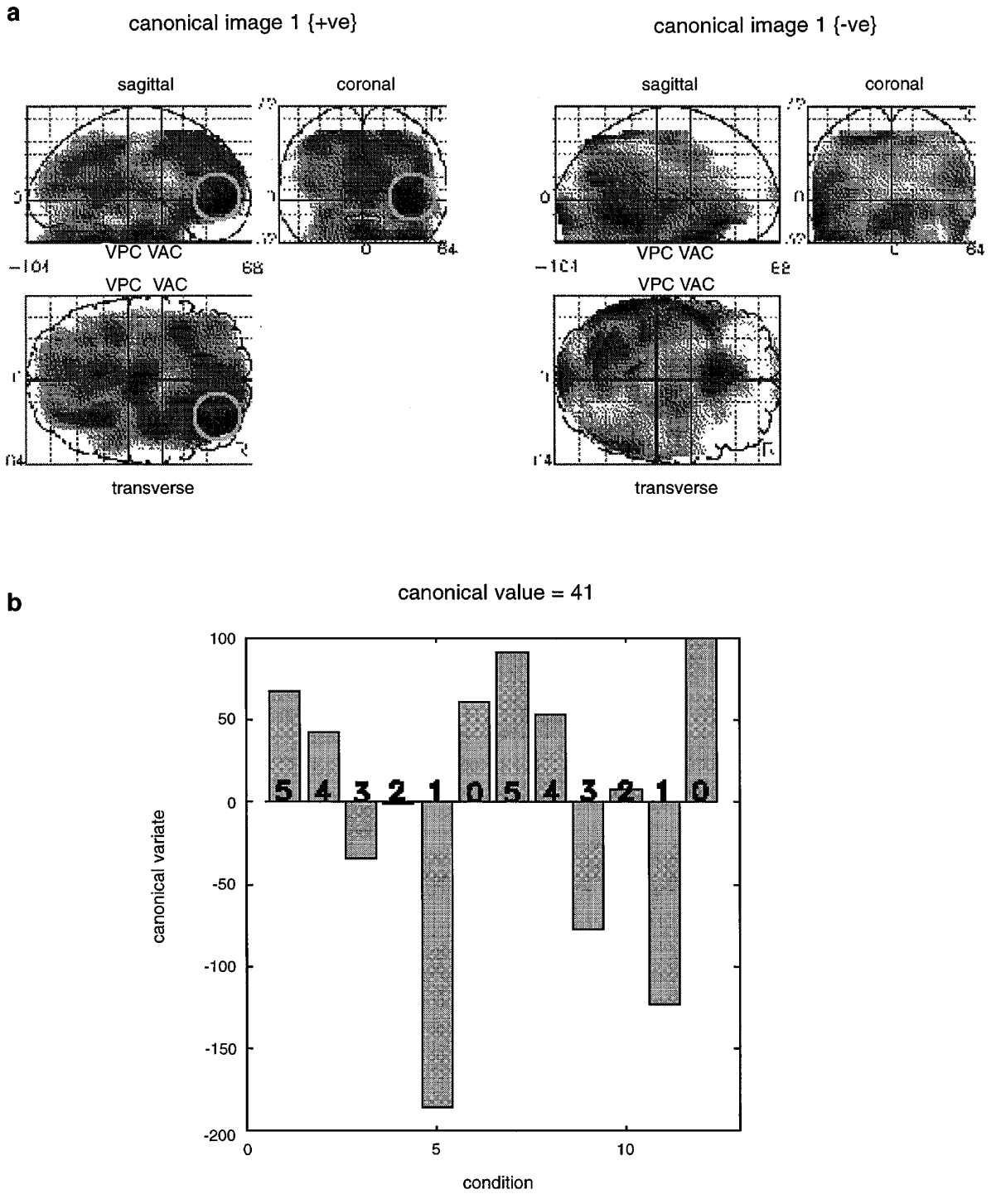

FIG. 3. (a) The first canonical image displayed as maximum intensity projections of the positive (left) and negative (right) components. Images are displayed as sagittal (from the right), coronal (from behind), and transverse (from above) views. The grayscale is arbitrary. The positive component of greatest intensity, the right prefrontal cortex, is encircled. (b) The expression of the first canonical image (i.e., the canonical variate) averaged over conditions. The first six bars represent the imageable recall conditions, and the last six represent nonimageable recall. Across each set of six conditions, strength of semantic linkage decreases from left (5) to right (0).

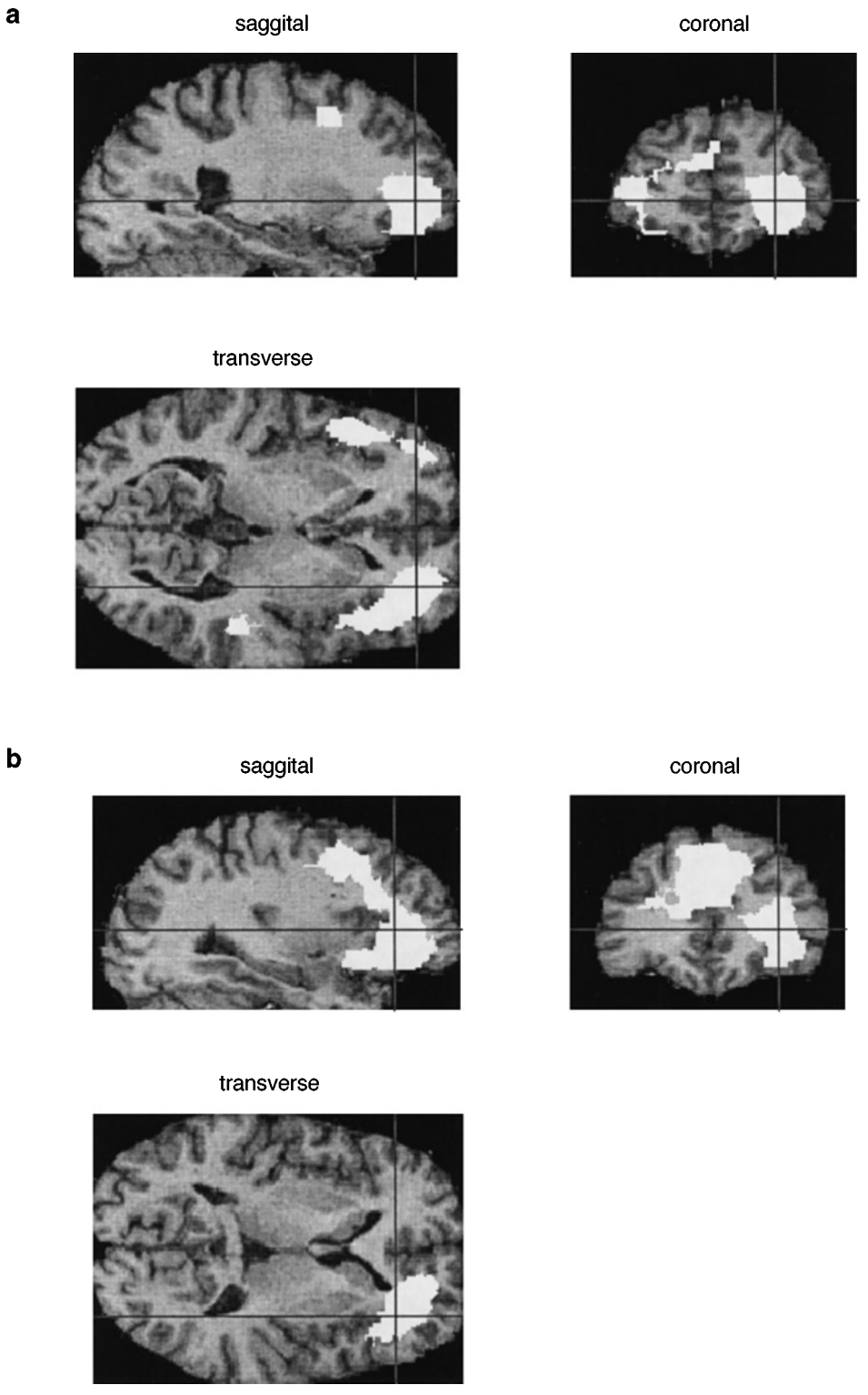

FIG. 4. Secondary analyses of the data following MANCOVA results. (a) Decreases in brain activity associated with a decreasing degree of semantic linkage between pair members. Scans obtained during the retrieval of unlinked pairs have been excluded. The decreases have been superimposed onto averaged MRI scans which have been normalized into a standard stereotactic space (Talairach and Tournoux, 1988). This demonstrates the anatomy more clearly. Medial and dorsolateral deactivations are seen bilaterally with a predominance on the right. (b) A direct comparison between scans obtained during recall of unlinked pairs with those obtained during recall of linked pairs. Results are presented as they are in a. The cross-sections show that unlinked pair recall is associated with marked frontal activation, predominantly medially and right dorsolaterally.

in frontal blood flow cannot be proposed as an explanation.

An alternative suggestion is that the frontal decrease does indeed reflect a change in monitoring/verification processing but, contrary to our initial presumption, the necessity for such processes decreases as the link weakens and is required in force once more when the semantic link is completely severed (semantic link = "0"). This suggestion is plausible if we consider that a response which is very closely linked to a cue (e.g., cue "*king*" . . . response "*queen*") is more likely to be automatically elicited and that this automaticity is not necessarily an aid to episodic memory. Indeed, we suggest that it may interfere with it—a subject who internally produces the correct response "*muscle*" in response to the cue "*arm*," can be more confident that this had not come to mind automatically (and erroneously) than when producing the response "*queen*" to the cue "*king*." In other words, it may be easier to verify that a more distant/less automatic response is correct, thus necessitating a lesser degree of right frontal activation. When the responses are no longer semantically linked with the cues, this facilitatory effect is lost, since, with the unlinked pairs, any response could be paired with any of the cues. Consequently, a greater degree of monitoring/verification would be required to ascertain that an internal response was indeed the correct one for the cue in question. This would be reflected in the increased right frontal activation that we observed.

In this description of our data, the important point is that the use of a multivariate analysis uncovered an unpredicted pattern of results. This pattern contributed most forcefully to the overall variance in the data but could have been overlooked using a standard analysis employing highly constrained hypotheses.

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