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BRIEF NOTE

The Inferential Impact of Global Signal Covariates in Functional Neuroimaging Analyses

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Neuroimaging techniques, such as PET and fMRI, are used to test hypotheses regarding local changes in neural activity in response to experimental manipulations. These changes are indirectly measured using raw counts or blood flow in the case of PET (Arndt *et al.,* 1996) or susceptibility in the case of BOLD fMRI (Ogawa *et al.,* 1993). Typically, data are acquired from the entire brain volume and analyses undertaken to identify subcomponents of this volume—voxels or regions of interest—in which significant signal changes have occurred (Friston *et al.,* 1995c).

Because neuroimaging experiments often test hypotheses regarding local changes in neuronal activity, variations in signal that are common to the entire brain volume (i.e., global blood flow in PET or global signal in fMRI) have been considered nuisance effects to be eliminated (Ramsay *et al.,* 1993). Therefore, the bulk of discussion to date concerning global signals has regarded the appropriate method of their removal (Fox *et al.,* 1988; Friston *et al.,* 1990; Arndt *et al.,* 1996). Specifically, the question of whether regional "activations" are proportional or additive to global signals has been debated at length, with the outcome of importance for scaling versus covariate approaches (Friston *et al.,* 1990). Using one or another of these techniques, numerous PET and fMRI studies in which the effects of the global signal have been removed have been reported (e.g., Cabeza *et al.,* 1997; Courtney *et al.,* 1996; Jonides *et al.,* 1993; Poline *et al.,* 1996; Schacter *et al.,* 1995; Vandenberghe *et al.,* 1996). Relatively unaddressed, however, is the underlying validity of adjusting for the effects of global signal changes in the first place. Specifically, it must be asked if global signals behave as confounds or simple nuisance variables in additive models (Friston *et al.,* 1995c).

Formally, confounding exists if "meaningfully different interpretations of the relationship of interest result when an extraneous variable is ignored or included in the data analysis" (Kleinbaum *et al.,* 1988). Confounding may be contrasted with a simple nuisance variable in that inclusion or exclusion of a confound will affect the expected relationship between the data and an independent variable of interest. In contrast, inclusion or exclusion of a simple nuisance variable will only affect the error variance of the model. In other words, covarying for confounds could change interpretation of statistical results both qualitatively (by changing the sign of relationships) and quantitatively (by either increasing or decreasing the significance of relationships), while covarying for simple nuisance variables could only quantitatively impact interpretation by increasing the significance of relationships (while preserving the sign). This difference in behavior between confounds and nuisance variables is due to correlation of the former with the independent variable(s) of interest. As global signals are spatial averages of the local signals of interest, which are themselves hypothesized to correlate with experimental treatments, it is reasonable to hypothesize that global changes would correlate with behavioral manipulations. If true, global signals will act as confounds, and hence interpretation of statistical results would be contingent upon whether a global signal covariate is either included or excluded.

PET count data reported by Friston and colleagues (1990) demonstrated a relationship of global flow with experimental condition that was large with respect to the magnitude of the adjusted local effect (see Fig. 6 from that report). Similarly, Aguirre and colleagues (1997) reported that there was a significant correlation between observed global fMRI signals and an experimental paradigm. These two results suggest that global neuroimaging signals can be correlated with the experimental manipulation and are thus not necessarily simple nuisance variables. The implication is that covarying for global signal in PET and fMRI analyses is not simply, or necessarily, increasing power, but meaningfully changing the results and hence interpretation of these studies.

To further address the issue of whether global neuroimaging signals could act as confounds, we analyzed an

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experimental fMRI dataset both with and without covariation for global signal (Friston *et al.*, 1990). If these two methods yielded qualitatively different results (as opposed to simply appearing to increase sensitivity), then we would reject the hypothesis that fMRI global signals are always nuisance variables in favor of the hypothesis that fMRI global neuroimaging signals can act as confounds.

During fMRI scanning (gradient echo echo-planar, TR = 2000 ms, TE = 50 ms) subjects made speeded, bilateral button presses in response to brief (500 ms) intermittent (every 16 s) changes in a central fixation cross (to a target circle of equal visual angle). Each of seven subjects performed 20 such trials. Further details regarding fMRI data acquisition and preprocessing can be found in Aguirre *et al.* (1998). Analysis was conducted within the framework of the general linear model, modified for serially correlated errors (Worsley and Friston, 1995; Zarahn *et al.*, 1997; Aguirre *et al.*, 1997). Data were transformed into Talairach space and spatially smoothed with a Gaussian kernel (FWHM = 15 mm). Independent variables were created using the first three principle components (eigenvectors) derived from a set of hemodynamic response functions from an independent group of subjects (Aguirre *et al.*, 1998). The first eigenvector resembles an across-subject, representative hemodynamic response function and it is the relationship between this covariate and the fMRI signal that was evaluated. SPM{*t*}s (Worsley, 1994) testing for evoked signals following the onset of the target were created for models both including and excluding global signal covariates (Aguirre *et al.*, 1997). A different global signal covariate was used for each subject, allowing the relationship of global to local signal to vary between subjects. Additionally, the relationship between the global signals themselves and the experimental paradigm was evaluated by concatenating the global signals obtained from the different subjects. This concatenated vector then served as dependent data for the model which did not include global signal covariates.

Figure 1a presents the thresholded SPM{*t*} corresponding to the first eigenvector (see above) for the model that lacked global signal covariates. As expected, local maxima corresponding to the experimental paradigm were found near the central sulcus as well as other locations. Of note, a large proportion of the observed brain volume had a significantly positive relationship with the task. Figure 1b presents the thresholded SPM{*t*} for the model that included global signal covariates. Several local maxima, particularly in the central sulcus and supplementary motor area, appear in both

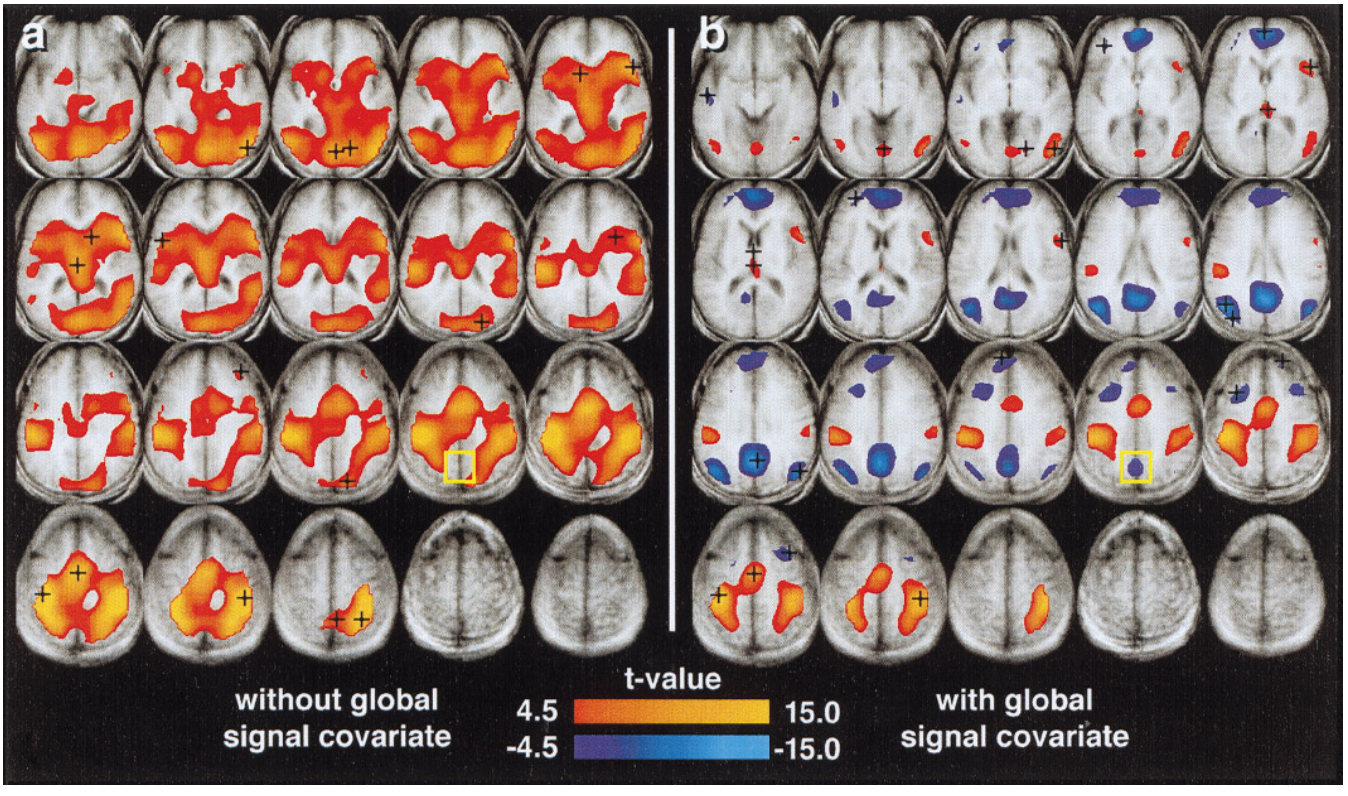

FIG. 1. SPM{*t*}s generated with (a) and without (b) global signal covariates. Local maxima are indicated with a black cross. The *t* threshold employed corresponds to a map-wise $\alpha = 0.05$ (two-tailed), corrected for multiple comparisons (Worsley, 1994). The yellow square indicates the region from which the trial-averaged signal presented in Fig. 2 was obtained.

Figs. 1a and 1b. Importantly, there were regions whose *t* value changed sign between Fig. 1a and Fig. 1b. This effect is well illustrated by Fig. 2, which shows the trial-averaged signal, adjusted for confounds (Friston *et al.*, 1995c), from the region outlined in yellow in Fig. 1b. When the model did not include a global signal covariate, the adjusted, trial-averaged signal displayed a positive relationship with the task. When, however, the data were adjusted (Friston *et al.*, 1995a) for effects of the global signal, a significantly negative relationship with the task was produced. Essentially identical results were obtained when proportional scaling at each time point, instead of a covariate approach, was used to correct for the effects of global signal change (data not shown).

A sufficient explanation of these dramatic effects can be posited based upon two observations regarding fMRI data. First, it has been previously demonstrated that in spatially smoothed data, even those collected without any experimental treatment (i.e., under null-hypothesis conditions), voxel time series from almost the entire cerebral volume are positively correlated with the global signal (Aguirre *et al.*, 1997). Second, an extremely significant, positive relationship between the global signal and the experimental paradigm [t(723 eff df) = 9.5, $P < 1 \times 10^{-5}$] was observed here. Given these features, consider a voxel time series that is weakly (or not at all) correlated with the experimental paradigm, but is correlated with components of the global signal that are unrelated to the experimental paradigm. Within the context of the general linear model, this time series is best explained by a positive loading upon the global signal covariate and a negative loading upon the task-independent variables. This mechanism is sufficient to explain the presence in Fig. 1b of several negatively correlated areas that did not have a significant relationship with the task in Fig. 1a. Such a mechanism would also lead to a general reduction in positively correlated voxels with the addition of a global signal covariate.

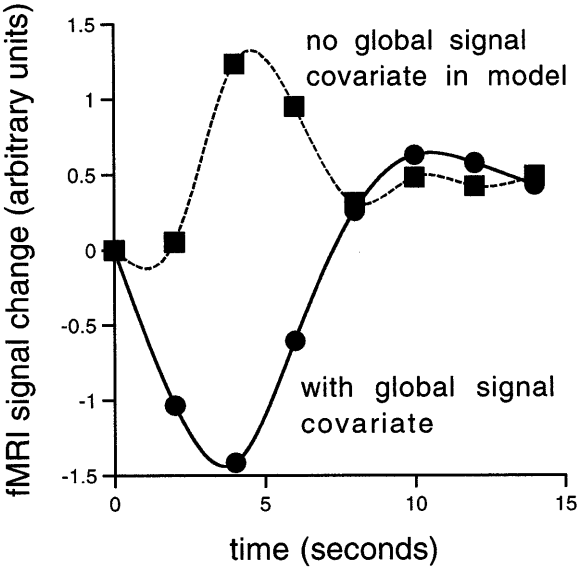

FIG. 2. Across-subject, trial-averaged, confound-adjusted, fMRI signal obtained from the area indicated by a yellow box in Fig. 1. The experimental paradigm required the subject to perform a speeded button press every 16 s. The curves presented here may be considered the regional, mean evoked hemodynamic responses that resulted from putatively brief periods of neural activity (which occurred at/around time zero). The upper curve (squares) did not have the effect of global signal removed and demonstrates a positive relationship with the experimental paradigm (i.e., a positive excursion in fMRI signal, which takes several seconds to evolve and decay, following isolated button presses). The lower curve (circles) was generated from the same data as the upper curve but has had the effect of the global signal removed. As can be seen, the inclusion of the global signal covariate in the model results in a negative relationship between evoked fMRI signal and experimental paradigm (see text for details).

It should be noted that spatial smoothing cannot account for the high correlation of the global signal with the task. Because the global signal is itself a spatial average, its shape is unaltered by any degree of spatial smoothing. Thus, the results presented here demonstrate that global signal covariates can act as confounds regardless of whether the imaging data are smoothed in space. However, spatial smoothing will tend to increase the correlation of voxel time series with the global signal (Aguirre *et al.*, 1997). Thus, these confounding effects will tend to be accentuated in spatially smooth data.

The strong, positive relationship observed here and elsewhere (Friston *et al.*, 1990; Aguirre *et al.*, 1997) between global signal and experimental paradigm disputes one of the assumptions regarding use of a global signal covariate (Friston *et al.*, 1990; Ramsay *et al.*, 1992), namely, that such a relationship does not exist. While the presence and/or magnitude of this relationship may vary from task to task or subject to subject, the present results demonstrate that it is at least possible for such a situation to arise. We have also shown that including a global signal covariate can meaningfully change results. We thus now raise the question of the inferential impact of the global signal covariate in hemodynamic functional neuroimaging studies. Consideration of Fig. 1a or 1b in isolation might lead one to very different conclusions regarding the cortical regions that display functional changes in response to a simple reaction time task. Three nonmutually exclusive mechanisms may be posited for the extensive activation seen in Fig. 1a (which was produced without a global signal covariate):

(1) Spatially discrete activation in conjunction with spatial smoothing could theoretically lead to mass activation in the resulting SPM. This, however, is not a

sufficient explanation in this case as it would require *t* values at the local maxima that were much larger than those observed here.

(2) There could have been a "nonspecific" cerebral hemodynamic change somehow (additively) superimposed on modest amounts of true local activation. Though not related to underlying local neural activity per se, it is important to understand that such a global change would have to be effected by the experimental treatment.

(3) It is possible that much of the brain did truly respond neurally to the experimental paradigm. There is no *a priori* reason to reject this prospect, which is perhaps surprising to those who subscribe to strict localizationist interpretations of brain function.

Despite the many reports examining different methods of controlling for global hemodynamic change, none has spoken to the relative contribution of mechanisms (2) and (3). If case (2) only is true, then it would be proper and necessary to somehow account for global signal when making inferences about brain function. The global signal in this case would be a confound that was truly impeding unbiased estimation of the effects of interest. If, however, case (3) [either on its own or in conjunction with case (2)] is true, adjustment for global signal would lead to inferences about brain function based on artifact. Specifically, the cases of artifact would consist of: (i) regions that actually have a relationship with the experimental paradigm will have this relationship reduced, possibly to levels below significance, and (ii) regions that have no relationship with the experimental paradigm will tend to manifest an apparent negative relationship with this independent variable.

In conclusion, it seems that when global signal covariates are included within an analysis, suprathreshold voxels should be regarded as those with a relationship that is significantly greater than that which the global signal enjoys. This implies that regions that evince a positive relationship with a task covariate in the *absence* of a global signal covariate will not necessarily evince a positive relationship with that task covariate in the *presence* of a global signal covariate. Furthermore, when a global signal covariate is present, it is inappropriate to automatically infer that regions with a negative relationship with the task have decreased signal values during control compared to experimental periods (for example), as this statistical circumstance may arise if the signal in a voxel is simply less correlated with the task than is the global signal itself. To allow for the proper interpretation of neuroimaging results, the degree of correlation of the global signal with the experimental paradigm should be reported in any study that employs global signal covariates (e.g., Aguirre and D'Esposito, 1997). Furthermore, in those cases in which the global signal is found to be significantly correlated with the experimental paradigm, it may be preferable to omit this confound as a covariate entirely.

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