On-line Appendix

SPM8 (Wellcome Department of Imaging Neuroscience, London, UK) preprocessed the qCBF and qCBV maps.[1] The field-echo-EPI DSC scan for each patient was spatially normalized to the standard MNI space EPI template. The normalized field-echo-EPI DSC scans were averaged to create a study-specific EPI template in MNI space.[2] The acquisition-space field-echo-EPI DSC scans and the intrinsically coregistered qCBF and qCBV maps were spatially normalized to the study-specific EPI template and resampled to $2 \times 2 \times 2$ mm. An isotropic Gaussian kernel (8-mm full width at half maximum [FWHM]) was applied.

The multispectral structural scans (ie, T1 and proton-attenuation [PD]/T2) for each patient were segmented into GM, WM, and CSF tissue probability maps using an automated validated algorithm.[3] WMLs were hand-traced on PD/T2 scans using Medical Image Processing, Analysis, and Visualization, Version 4.0 (National Institutes of Health, Bethesda, Maryland) and algorithmically removed from GM and WM tissue maps to correct potentially misclassified parenchymal tissue. The corrected GM, WM, and WML tissue maps were spatially normalized to MNI space using Diffeomorphic Anatomical Registration Through Exponentiated Lie Algebra registration (http://brainmap.wisc.edu/pages/8-Normalizing), resampled to $1.5 \times 1.5 \times 1.5$ mm, modulated, and smoothed using an isotropic Gaussian kernel (8-mm FWHM).

qCBF and qCBV maps were analyzed using the mass univariate methodology of SPM8.[1] Voxel-by-voxel *t* tests were performed to identify focal differences in qCBF and qCBV between cognitively impaired and nonimpaired patients. Global normalization was achieved by proportional scaling of the thresholded mean voxel value of every qCBF or qCBV map to a common value. We reported significance using a voxel-wise *P* value threshold ($P < .05$) corrected for multiple comparisons and an extent threshold of 20 contiguous voxels. The correction methodology uses random field theory to control the FWE rate, which represents the type I error rate in the family of voxels as a whole.[4] FWE-based correction is known to be conservative, so the spatial extent of each significant cluster was established using an uncorrected *P* value threshold ($P < .001$).[5] Extracranial voxels were excluded from analysis with a whole-brain mask. Using the WFU Pick Atlas, Version 3.0, toolbox for SPM8, we classified every voxel in each significant cluster according to cytoarchitectonic, lobar, and hemispheric location.[6]

Voxel-by-voxel *t* tests were also conducted to detect focal differences in GM, WM, and WML tissue volume between cognitively impaired and nonimpaired patients. Total intracranial volume was included as a statistical covariate to assess relative tissue volume. Global GM, WM, and WML volumes (cubic millimeters) were calculated by thresholding the respective tissue probability maps at .05. These values were normalized to total intracranial volume. Such normalized tissue volumes are more informative than absolute tissue volumes because the normalization step negates head size as a possible confounder. Normalized GM, WM, and WML volumes were analyzed using the Wilcoxon rank sum test.

qCBF and qCBV maps were further analyzed with PLS (Rotman Research Institute, Toronto, Ontario, Canada) using a multivariate methodology.[7] While SPM is designed to detect spatially localized differences in mean voxel intensity (within the context of the 2-group comparisons described above), PLS is designed to detect spatially distributed patterns of interdependency between voxel intensities and behavioral performance (within the context of Behavior PLS). LVs were derived from the correlation matrix of voxel and behavioral data by singular value decomposition. Each LV is associated with a singular value that is the covariance between voxel and behavioral saliencies with more predictive LVs having higher singular values. Behavioral performance represented dichotomized group membership (ie, impaired versus nonimpaired) for the patient overall (ie, $\geq$2 test impairments), which is similar to the 2-group SPM strategy, and for each cognitive test. Clinical and radiologic data found to be significant were included as "behavioral data," which consequently included all predictive variables.

The statistical significance of identified LVs was determined using permutation testing with 500 iterations.[7] For each permutation, PLS analysis was recalculated after randomly exchanging labels on data points, which is sampling without replacement. The frequency with which the permuted singular values exceeded the observed singular value was used to calculate the *P* value for the associated LV. The reliability of voxel saliencies was assessed using bootstrap resampling with 100 iterations.[1] Voxels were considered reliable if the ratio of their salience to standard error, which is referred to as the bootstrap ratio, was >5. A bootstrap ratio > 5 approximately corresponds to $P < .0001$, which represents the lower estimable limit of significance. Bootstrap ratio maps thresholded at >5 with an extent threshold of 20 contiguous voxels were used to identify significant clusters. Because image-wide statistical analysis is performed in a single step, no multiple comparison correction is necessary. The MNI space coordinates of significant voxels were converted to Talairach space using the icbm_spm2tal algorithm and then were classified according to cytoarchitectonic, lobar, and hemispheric locations using the Talairach Daemon database (http://www.talairach.org/daemon.html).[8,9] Correlations were calculated between predictive variables and the observed patterns of qCBF and qCBV. The 95% confidence intervals not overlapping zero indicated significant contribution to these patterns.

References

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