

We congratulate the author for this excellent article, which offers important methods and ideas for inference on functional Magnetic Resonance (fMR) images, with particular application to identifying and testing for theories on working memory utilization. Developing statistical methodology for the analysis of fMR images is an exciting and challenging task, bringing together issues from diverse disciplines such as neurology and psychology and we believe that this article offers a substantial breakthrough in this regard. Our main comments pertain to some of our recent experiences with fMR experiments which reveal issues of statistical import, some of which may be approached by extending the author's techniques.

The blood-oxygenation-level-dependent (BOLD) contrast referred to in the paper uses the difference in MR signals between oxy- and deoxy-hemoglobin levels that are caused by the excess inflow of blood due to neural activation. The differences in signals are very slight, usually on the order of 2–5 per cent, depending on magnetic field strength. Unfortunately, there is substantial recent evidence (Lai, *et al.*, 1993; Haacke *et al.*, 1994; Segebarth *et al.*, 1994) to suggest that such differences can also arise due to the presence of draining veins in the cortex. Figure 1 illustrates this in the context of a fMR experiment where the goal was to identify cortical regions of activation in a visual task experiment. Note that the areas of significant activation (Figure 1a) and the underlying macroscopic vascular structure, as depicted through a magnetic resonance angiogram (MRA) (Figure 1b) show substantial overlap,

as further seen by the superposition of the activation image on the MRA (Figure 1c). (An angiogram is a MR imaging technique made sensitive to regions with high blood flow rates — of at least 10 cm/s in our experiment.) This causes the concern that the predominance of extra-cerebral signals from these macroscopic vessels may provide inaccurate identification (in terms of size, shape and spatial location) of regions of neural activation. Further, there is inter-subject variability in the association of activation patterns with vascular structure (Segebarth *et al.*, 1994) which should be accounted for, especially in block-level multi-subject studies. Suppression of vascular signals has been attempted using a variety of approaches (see for example Bandettini *et al.*, 1994; Haacke, *et al.*, 1994; Hlušík, *et al.*, 1998) ranging from using a different imaging pulse sequence to post-processing, but we wonder whether this can be accounted for in an integrated framework by incorporating this information into the mean baseline signal μ of the author's Bayesian time-course model. Thus, instead of modeling μ independently on each voxel, one could use the MRA to specify a prior which would incorporate the vascular structure into these μ 's. It may be possible to do this by modifying the approach of Johnson, *et al.* (1995) mentioned by the author in the discussion. In particular, since veins are thin continuous structures, a line process may be an appropriate choice for modeling prior belief. Additionally, one may also adopt a strategy similar to the one used by the author to test for contribution of vascular artifacts in contributing to signal differences.

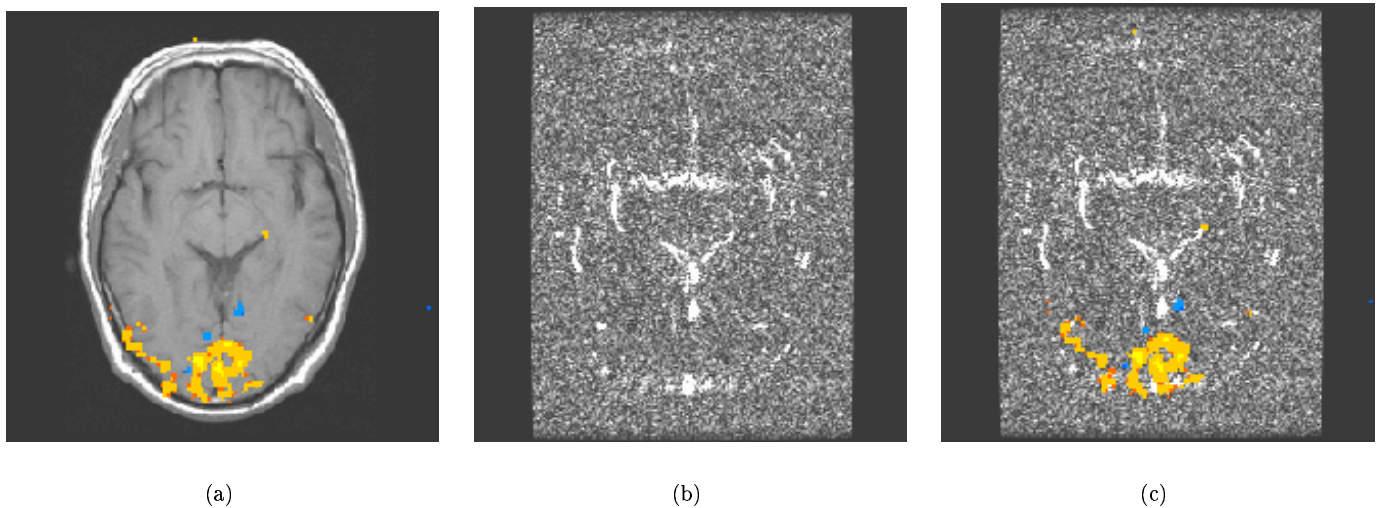


Figure 1. (a) Activation map of the visual cerebral cortex during an fMR imaging experiment, (b) angiograph image of the brain, indicating draining vessels, and (c) activation regions superposed onto the angiograph image, indicating that regions of significant activation overlap in areas of vascularity.

Our next comment concerns the slice prescription used in the article. The author uses seven slices that are each 5 mm thick and 1 mm apart. In general, one needs to register the slices before analyzing the fMR data. Typically, a three-dimensional rigid body registration technique (Woods *et al.*, 1998) is employed for this purpose. The sets of image slices are translated and rotated in all three planes to obtain the best match between the corresponding voxels. One criterion for such a match is the minimization of the least-squares differences in voxel intensity values. A further step called re-slicing is needed to get back the original number of slices in the image (which gets distorted as a result of the registration) for further analysis. With an inter-slice gap of 20% of slice thickness as used in the article, interpolation steps are needed before the best match minimization step. When signal differences are on the order of 2–3% such interpolation could lead to erroneous results and the identification of “false positives”. Indeed, studies (Noll *et al.*, 1997) have shown improved performance when there is no inter-slice gap. It is not clear why data on contiguous slices were not acquired, and it would seem that applying the author’s Bayesian time-course model on such data would produce even better results than those reported by the author in this article.

Our third comment pertains to the presence of motion artifacts in the fMR images, especially in the context of testing for monotonically increasing demands of the working memory experiment. The author corrects data for motion effects and performs registration using the public-domain software package FIASCO. The success of his methodology is limited by the ability of this and other similar motion-correcting algorithms in removing any voluntary or involuntary motion within the given time-series at any voxel. This is because signal changes that are on the order of 2–3% and due to the differences in oxy- and deoxy-hemoglobin levels in the blood can be easily overwhelmed by the residual motion or “paradigm-correlated motion”. Constraining the subject’s movement by means of restraints such as foam rubber head clamps (Burton and Small, 1999) may have the unintended consequence of making the subject uncomfortable, and thus interfere with the goals of the actual activation experiment. Our experience with standard algorithms has been that these perform reasonably well when the movement is very slight, failing which several “false positives” show up. It would therefore be of great practical significance to the investigator if there is a testing mechanism to decide when the acquired motion-corrected fMR data are good enough for testing for activation. This is a data quality issue where the goal is to determine whether the experiment has provided a dataset that can be used to make reasonable inference or whether the dataset is so contaminated with motion artifacts that it is better to reject the dataset from the experiment and to acquire a new dataset. Of course, such an approach would only be applicable when making inferences using normal subjects and not in a clinical study where the motion may be due to some biological disorder suffered by the patient.

Our final comment address the issue of visualizing the activation maps. Typically, the activation maps as deter-

mined by correlation analysis or *t*-tests (Bandettini *et al.*, 1993) are overlaid on structural images. Although these structural images are obtained from the same location as the functional images, they are obtained at a different time and hence there exists the possibility that the subject may move between these two scans. The contrast available in the functional image is completely different from that available in the structural image and hence it is difficult to register these two images. This leads to potential mis-mapping of the functional activation on the structural image. The problem is further exacerbated when the underlying structural images are obtained using a three-dimensional MR imaging technique, in which case the slice thickness, orientation and even the planes between the functional and structural images may not match. Developing techniques that would overcome such mis-mapping would greatly benefit clinicians and provide them with useful tools for pre-surgical planning.

In conclusion, the author has formulated a very promising approach in an important and challenging area. However, as demonstrated above, there are several related issues that arise and we hope that this paper will generate interest and further spur the development of methodology to settle statistical issues of practical significance.

ADDITIONAL REFERENCES

- Bandettini, P. A., Wong, E. C., Jesmanowicz, A., Hinks, R. S., and Hyde, J. S. (1994), “Spin-echo and gradient-echo EPI of human brain activation using BOLD contrast: a comparative study at 1.5T,” *Nucl. Magn. Reson. Biomed.*, 7, 12-20.
- Burton, M. W., and Small, S. L. (1999), “An introduction to functional magnetic resonance imaging,” *The Neurologist*, 3, 145-58.
- Haacke, E. M., Hopkins, A., Lai, S., Buckley, P., Friedman, L., Meltzer, H., Hedera, P., Friedland, R., Klein, S., and Thompson, L. (1994), “Decomposition of inflow and blood oxygen level-dependent (BOLD) effects with dual-echo spiral gradient-recalled echo (GRE) fMRI,” *Nucl. Magn. Reson. Biomed.*, 7, 54-62.
- Hlušík, P., Noll, D. C., and Small, S. L. (1998), “Suppression of vascular artifacts in functional magnetic resonance images using MR angiograms,” *Neuroimage*, 7, 224-231.
- Lai, S., Hopkins, A. L., Haacke, E. M., Li, D., Wasserman, B. A., Buckley, P., Friedman, L., Meltzer, H., Hedera, P., and Friedland, R. (1993), “Identification of vascular structures as a major source of signal contrast in high resolution 2-D and 3-D functional activating imaging of the motor cortex at 1.5T: preliminary results,” *Magn. Reson. Med.*, 30, 387-92.
- Noll, D. C., Boada, F. E., and Eddy, W. F. (1997), “Movement correction in fMRI: The impact of slice profile and slice spacing,” *Proceedings of the International Society for Magnetic Resonance in Medicine, Fifth Scientific Meeting and Exhibition*, Vancouver, Canada, 1677.
- Segebarth, C., Belle, V., Delon, C., Massarelli, R., Decety, J., Le, B. J. F., Decors, M., and Benabid, A. L. (1994), “Functional MRI of the human brain: predominance of signals from extra-cerebral veins,” *Neuroreport*, 5, 813-16.
- Woods, R., Grafton, S., Holmes, C., Cherry, S., Mazziotta, J. (1998), “Automated image registration. I. General methods and intra-subject, intra-modality validation,” *Jour. Comput. Assist. Tomogr.*, 22, 141-54.

Ranjan Maitra is Assistant Professor in the Department of Mathematics and Statistics, University of Maryland, Baltimore County, Baltimore, MD 21250 (E-mail: maitra.math.umbc.edu) and Rao Gulapalli is Assistant Professor in the Department of Radiology, University of Maryland School of Medicine, Baltimore, MD 21201 (E-mail: rpg@rad1.ummc.umaryland.edu).