

Discrimination of Errors From Neuronal Activity in Functional MRI of the Human Spinal Cord by Means of General Linear Model Analysis

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Functional MRI (fMRI) of the spinal cord has been demonstrated to provide reliable and sensitive maps of neuronal activity, particularly when combined across several experiments. Individual experiments reveal neuronal activity as well as errors. The dominant source of errors is hypothesized to be physiological motion, including cardiac and respiratory motion, flow of blood and cerebrospinal fluid (CSF), and motion of the spinal cord within the spinal canal. All of the hypothesized sources of error are therefore related to cardiac and respiratory motion, which can be recorded during an fMRI experiment. Analyses were carried out with a general linear model (GLM) with peripheral pulse and respiration recordings used as models of errors. The results demonstrate that the sensitivity of spinal fMRI is improved and errors are reduced when peripheral pulse traces are used in the GLM, but no improvement was detected with the inclusion of respiratory traces. Magn Reson Med 56:452–456, 2006. © 2006 Wiley-Liss, Inc.

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Functional MRI (fMRI) of the spinal cord has been shown to reliably demonstrate activity in response to a variety of noxious and innocuous sensory and motor stimuli (1–5). Studies have been carried out in healthy volunteers, as well as in subjects with spinal cord injury or multiple sclerosis (6,7). These studies have revealed a wealth of information about the activity that can be detected in the spinal cord, the magnitude and underlying mechanisms of neuronal-activity-related signal intensity changes, and how problems of susceptibility differences, poor field homogeneity, small anatomical dimensions, and motion can be overcome or reduced (1,8). However, the demonstrated reliability has been obtained primarily in combined results across subjects or repeated studies. The reliability of individual experiments is lower because of the occurrence of Type I errors (voxel shown to be active, in error) and Type II errors (voxel shown to be not active, in error). The goal of the present study was to identify the source(s) of these errors and to reduce or eliminate their effect.

The dominant source of errors in spinal fMRI is hypothesized to be physiological motion arising from the flow of blood and cerebrospinal fluid (CSF), bulk cord motion, and breathing. CSF flow and/or blood flow drive the motion of the cord, and these are in turn driven by the heart. This means that the origins of the physiological motion are all related to either the heartbeat or respiration. The effects of temporal magnetic field variations that can arise from magnetic susceptibility differences around the heart and lungs are expected to be of minor significance because the spinal fMRI method is designed to be relatively insensitive to susceptibility differences in order to obtain high-quality images of the spinal cord. Nonetheless, these effects would also correspond with the motion of the heart and lungs. It has been shown that cardiac-gated acquisitions improve the sensitivity of spinal fMRI results (9); however, eliminating the signal from CSF by means of inversion-recovery methods and spatial saturation has not been demonstrated to be of any benefit (10). Although it is well known that the spinal cord moves within the spinal canal, and this movement has been measured to be relatively small (~1 mm) (11), the pattern and origins of the motion have not yet been determined.

The purpose of this study was to investigate the origins of the errors in spinal fMRI by modeling the suspected sources and eliminating their contribution, if these are indeed sources of error, by performing an analysis with a general linear model (GLM) (12). It is hypothesized that the apparent signal intensity fluctuations that give rise to errors are caused by spinal cord motion and can be modeled from recordings of the heartbeat and/or the respiratory motion. If there is a significant component of the signal-intensity time course that matches the cardiac and/or respiratory motion, one could conclude that this motion, or that imparted to the CSF, blood, or spinal cord itself, is a significant source of errors.

MATERIALS AND METHODS

Spinal fMRI studies were carried out on a 3 T Siemens Magnetom Trio using a phased-array spine receiver coil and posterior neck coil, with the subjects lying supine. The appropriate receiver coil elements were selected for imaging the volume of interest. Ten healthy volunteers were studied and provided informed consent prior to participating. One volunteer was studied twice on separate days, for comparison. Initial localizer images were acquired in three planes as a reference for slice positioning for subsequent fMRI studies. The functional imaging data were acquired with a half-Fourier single-shot fast spin-echo sequence (HASTE) with the echo time (TE) set at

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38 ms, and repetition time (TR) of 1.5 s per slice. Signal intensity changes observed upon a change in neuronal activity were the result of signal enhancement by extravascular water protons (SEEP), as well as a contribution from the blood oxygenation level-dependent (BOLD) effect, as described previously (13,14). Sagittal image data were acquired from seven contiguous sagittal slices, each 2 mm thick, centered on the C7 vertebra, with an 18 cm \times 9 cm FOV and a 192 \times 96 matrix. The resulting voxel size was 0.94 mm \times 0.94 mm \times 2 mm. Spatial suppression pulses were applied to eliminate signal from anterior to the spine, and flow-compensation gradients were applied in the rostral-caudal direction. The peripheral pulse and respiration were recorded continuously during each study using the physiological monitoring equipment that is incorporated into the Siemens MRI system. A time reference was provided by means of an external trigger, generated by a National Instruments data acquisition board (DAQpad-6020E), which was input to the MRI hardware, recorded synchronously with the physiological traces, and used to trigger the acquisition of each image.

Cold thermal stimulation of the heel of the thumb on the palm of the right hand was used to elicit activity in the cervical spinal cord by means of a Medoc® TSA-II thermal sensory analyzer. In each experiment the same cold stimulation temperature and duration of stimulation were applied three times, separated by periods at the baseline temperature of 32°C. In every case the temperature transition spanned a period of 10.5 s. In separate experiments the stimulation temperature was applied at either 15°C or 25°C, and was applied for a duration of either 63 s or 21 s. This experimental design was chosen to provide a range of responses for testing the impact of the GLM analysis.

The resulting three-dimensional functional image data were analyzed as described previously (8) by first drawing a reference line along the anterior edge of the cord in a mid-line slice. The data were then reformatted to cubic voxels (0.94 mm in each direction) by means of bilinear interpolation, and resliced perfectly transverse to the cord at each position in the rostral-caudal direction. Smoothing was then applied only parallel to the long axis of the cord in order to smooth only within consistent tissues. The data were then analyzed using a GLM ($P < 0.01$) with basis sets composed of a boxcar model paradigm as described above, a constant function to account for the baseline intensity, and a linear ramp function to account for baseline trends. This basis set was used for one analysis, and analyses were repeated with peripheral pulse traces, respiratory traces, or both included in the basis set. However, the phase of the cardiac or respiratory cycles was different at the time of acquisition of each slice, and the phase of the motion of the cord relative to the heartbeat or respiration (and whether they are related) is unknown. The basis sets were therefore constructed with the aliased physiological traces for each slice, and the traces for all slices were used for the analysis of every voxel. This approach takes into account the unknown phase of the motion that contributed to each slice. The set of physiological traces was then reduced to its principal components and included in the basis set for analysis with the GLM. The model paradigm was not included in the set of basis functions that was reduced to principal components, because it is necessary

to be able to determine the specific contribution from the model paradigm in the observed signal intensity time-courses.

Consistency of active regions across subjects was determined by coaligning the results from each subject and constructing binary maps of activity (1 = active, 0 = not active). These were dilated so that clusters or individual active voxels were extended by 1 voxel in every direction and then summed across all subjects. The result demonstrates the number of subjects that showed activity at each voxel, or in an immediately neighboring voxel to allow some leeway for imperfect alignment and anatomical variability.

RESULTS

Activity was detected in all 10 volunteers (11 experiments), and the activity was consistent across subjects, as shown in Fig. 1. A schematic of the cord anatomy, in the orientation used in all other figures, is shown in Fig. 2. Analysis based on the GLM was observed to exclude or reduce some areas of Type I errors as identified by being outside of the spinal cord, and resulted in the identification of more active voxels within the cord. Figure 1 (top panel) demonstrates the consistent activity at approximately the level of the sixth cervical spinal cord segment with noxious thermal stimulation at 15°C, for 63 s and 21 s in comparison, as detected with the four different basis sets used in the GLM. Corresponding results for the same region of the spinal cord are shown in Fig. 1 (bottom panel) for thermal stimulation at 25°C for 63 s and 21 s in comparison. The maps of activity shown in Fig. 3 demonstrate the consistent areas of activity as in Fig. 1, except that areas outside of the spinal cord are included. The areas outside the cord cannot represent true activity, and therefore they represent false activity (Type I errors). Figure 3 demonstrates differences in the quantity of these Type I errors with the four different basis sets used for analysis.

In most subjects, most of the activity was observed at approximately the level of the sixth cervical spinal cord segment (C6) in the dorsal horn gray matter (GM), ipsilateral to the stimulus. Activity was more consistently detected and spanned more voxels with the painful stimuli (15°C) than with the nonpainful stimuli (25°C, Fig. 1). The activity with the painful stimuli was more superficial, near the tip of the dorsal horn, and was accompanied by activity in the ventral (motor) areas, as well as in the contralateral dorsal GM. Activity was also consistently detected in the upper cervical spinal cord at the second or third spinal cord segments (C2/C3), ipsilateral to the stimulus, with the painful stimuli, and contralateral to the stimulation with nonpainful thermal stimuli. With the painful stimuli the activity in ventral GM regions was both ipsilateral and contralateral, whereas with the nonpainful stimuli the ventral activity was only ipsilateral, spanned fewer voxels, and was less consistent across subjects. Activity in the ipsilateral dorsal GM (sensory areas) was more consistently detected with the nonpainful stimulus with a short duration of 21 s than with the longer duration of 63 s, which may reflect some adaptation of the thermal receptors on the hand to the stimulation temperature.

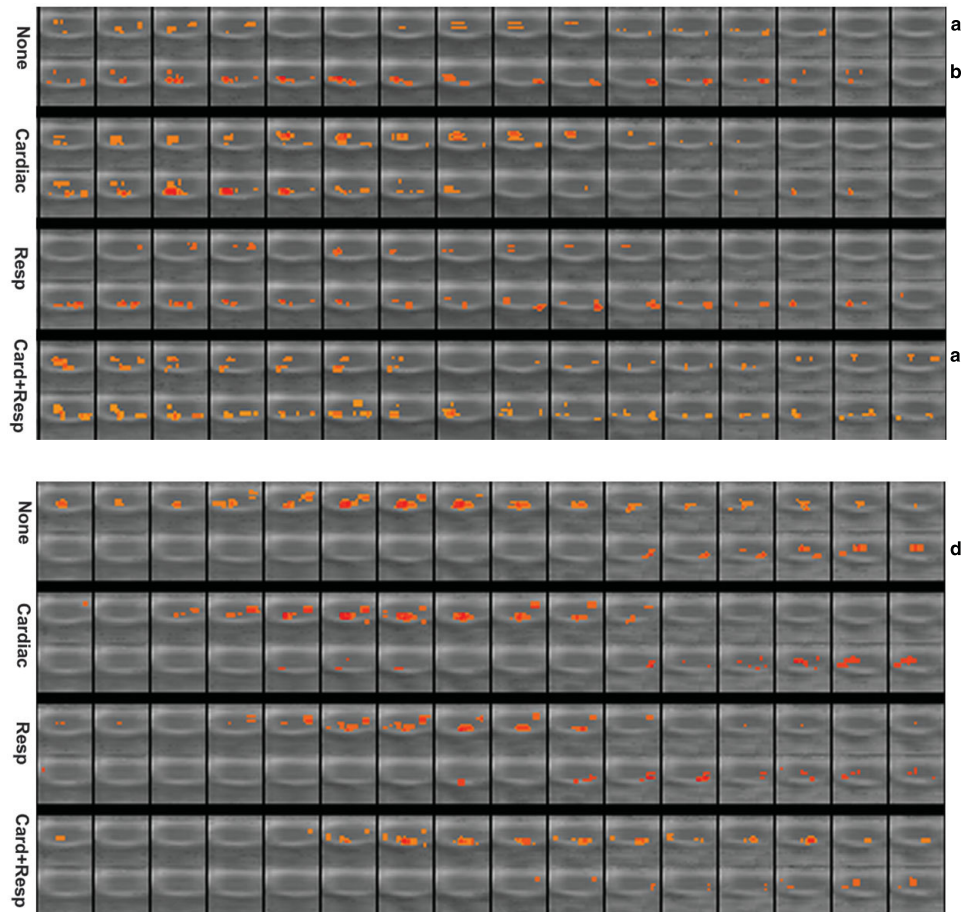


FIG. 1. Consistent activity across 16 reformatted transverse slices (1 mm thick) at approximately the level of the sixth cervical spinal cord segment, with noxious thermal stimulation. Stimuli were applied at 15°C for 63 s (a) or 21 s (b), or at 25°C for 63 s (c) or 21 s (d). Individual results were aligned based on the positions of the dorsal nerve roots exiting the eighth cervical spinal cord segment. Each set shows voxels that were identified as being active in at least three of the 11 experiments. The four sets represent analysis with the GLM with no physiological recordings included in the basis set (top set), with cardiac traces included (second from top), with respiratory traces included (third from top), and with both cardiac and respiratory traces included in the basis set (bottom set). Images are in radiological orientation with the right side of the body toward the left of the image, and dorsal is toward the bottom.

DISCUSSION

Comparisons of activity maps obtained in the spinal cord with GLM analysis demonstrated results that were consistent across subjects and with the spinal cord neuroanatomy, but there were significant differences depending on the basis sets used. Interpretations of whether areas of activity represented true-positive results or errors can be made based on the consistency with the neuroanatomy for a given stimulus, and the differences between stimuli, as well as the consistency across human subjects. Activity is expected with all thermal stimuli in the ipsilateral dorsal GM, and motor reflex activity is expected in more ventral GM both ipsilateral and contralateral to the stimulus. Some activity in the contralateral dorsal GM is also expected to arise with painful thermal stimuli because of descending input from the periaqueductal GM. The rostral-caudal level of the activity in the spinal cord GM is expected to be fairly consistent within each subject for all of the stimuli applied in this study because the stimuli were applied to the same part of the palm of the hand. In each study, experiments were carried out in the same

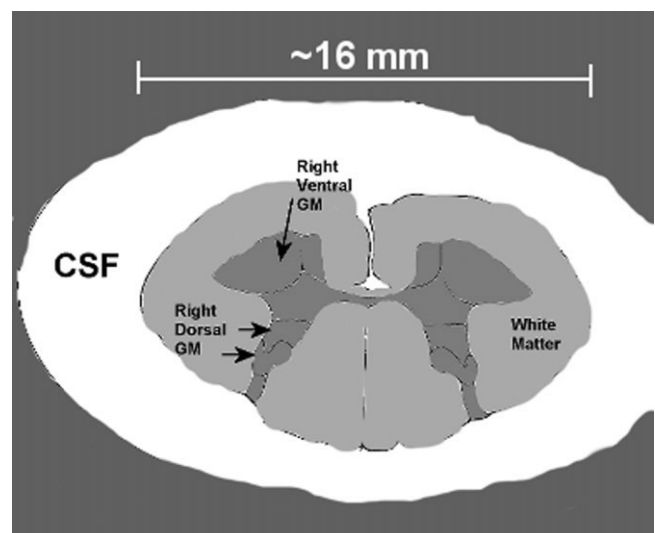


FIG. 2. Schematic illustration of the cervical spinal cord anatomy and surrounding CSF, as oriented in the results shown in Figs. 1 and 3.

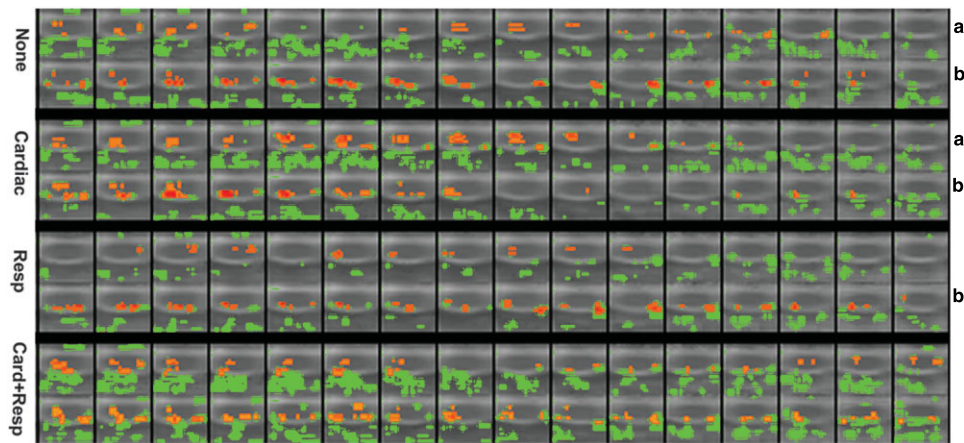


FIG. 3. Comparison of the first two experiments with noxious thermal stimuli at 15°C, applied for 63 s (a) or 21 s (b), at the level of the sixth cervical spinal cord segment. Images are reformatted 1-mm-thick slices, transverse to the spinal cord. Each set shows voxels that were identified as being active in at least three of the 11 experiments. Activity shown in red/orange falls within the spinal cord, whereas activity shown in green is outside of the cord and cannot be attributed to neuronal activity and therefore represents a Type I error. The four sets represent analysis with the GLM with no physiological recordings included in the basis set (top set), with cardiac traces included (second from top), with respiratory traces included (third from top), and with both cardiac and respiratory traces included in the basis set (bottom set). Images are in radiological orientation with the right side of the body toward the left of the image, and dorsal is toward the bottom.

order: 1) 15°C for 63 s, 2) 15°C for 21 s, 3) 25°C for 63 s, and 4) 25°C for 21 s. Variations in activity that may be caused by previous stimuli or sensory adaptation are expected to be similar across subjects. Therefore, the consistent areas of activity across subjects are expected to reveal the true areas of activity, and higher consistency and greater sensitivity with a particular analysis method should be demonstrated by greater consistency in the results across subjects. Areas that are clearly identified as false activity (Type I errors) are any areas of apparent activity that lie outside of the spinal cord.

Based on the patterns of consistent activity observed in the cervical spinal cord with the four different stimuli applied in this study, as shown in Fig. 1, the results clearly demonstrate that the use of the GLM alters the areas of activity that are detected, and changes the amount of false-positive results observed outside of the spinal cord. More areas of activity were observed in the spinal cord with the peripheral pulse traces used as the basis set, or with the combination of both peripheral pulse and respiratory traces. This indicates that the number of truly active voxels that were misidentified (Type II errors) was reduced. With the respiratory traces alone used as the basis set, the areas of activity were observed to be slightly different, but no general trend of increased activity was observed, and no trend of increased consistency was observed. When both peripheral pulse and respiratory traces were incorporated into the basis set, more activity observed, but it was more scattered, and more false-positive activity outside of the cord was observed. This combination of physiological traces in the basis set appears to introduce considerably more Type I errors. The use of the peripheral pulse traces alone demonstrated more active voxels, with greater consistency across subjects and more consistency with the stimuli and anatomy. Of the four basis sets used for the GLM analysis, the inclusion of the peripheral pulse traces alone was observed to produce the highest degree of sensitivity and reliability.

Combined activity maps showing the consistency of results on a voxel-by-voxel basis demonstrated clear patterns of activity with each stimulus when analyzed with peripheral pulse traces included in the basis set of the GLM. With 15°C thermal stimulation applied for both 63 s and 21 s (Fig. 1, top panel), activity was clearly observed at the tip of the ipsilateral dorsal horn at roughly the sixth cervical spinal cord segment, across a range of several millimeters, consistent with painful stimulation. With both of these stimuli, activity was also consistently demonstrated in ipsilateral motor areas related to the reflex to withdraw from the sensation. Individual results demonstrated consistent activity in the ipsilateral dorsal horn at the level of the sixth cervical spinal cord segment in nine of the 11 experiments, demonstrating a high degree of reproducibility. Stimulation at 25°C for 63 s or 21 s (Fig. 1b) also demonstrated activity at the sixth cervical spinal cord segment, but the activity was more localized. No consistent activity in motor areas was observed that could reliably be attributed to a reflex to withdraw from the sensation. This result is expected with an innocuous stimulus, but has never before been tested with spinal fMRI. Activity was also more clearly demonstrated in the ipsilateral dorsal horn with the briefer stimulus of 21 s, as compared to the longer stimulus of 63 s. This difference may represent a decrease in sensitivity with the longer stimulation because of adaptation of the thermal receptors to the temperature. With both durations of stimulation at 25°C, activity was observed in the upper cervical spinal cord but was contralateral to the side of stimulation, in contrast to that observed with stimulation at 15°C. These results demonstrate that a high degree of sensitivity was achieved with the GLM analysis that included the peripheral pulse traces as a component of the basis set.

The observation that the sensitivity was increased and the amount of Type I errors was decreased with the use of peripheral pulse traces in the basis set indicates that a significant source of errors in spinal fMRI is related to the

cardiac cycle, and is consistent with the observations of Brooks et al. (9). Moreover, similar improvements were not observed with the use of respiratory traces in the basis set. Including both respiratory and peripheral pulse traces in the basis set appeared to produce more Type I errors. The detection of more false-positive results indicates that instead of separating errors from neuronal-activity related signal changes, the basis set used in the GLM in this case was not adequately designed to discriminate inactive from active voxels. This can occur if the basis set is not composed of independent functions, and presents a limitation of the approach used in this study. This problem may be eliminated by including only a small number of accurate models of the spinal cord motion in the basis set, instead of modeling to account for the unknown phase of the motion.

CONCLUSIONS

The sensitivity and reliability of spinal fMRI results are significantly improved with analysis based on a GLM with a basis set that includes traces of the peripheral pulse sampled at the frequency of the image acquisition. The results demonstrate that both Type I and Type II errors are reduced with this method. No specific improvements were detected with the use of respiratory traces in the GLM. The findings of this study demonstrate that motion related to the cardiac cycle is a significant source of errors in spinal fMRI.

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