Spinal Cord (2007) 45, 485–490

www.nature.com/sc

Original Article

Detection of the neuronal activity occurring caudal to the site of spinal cord injury that is elicited during lower limb movement tasks

J Kornelsen[1] and PW Stroman*[,2]

[1]*Department of Physiology, University of Manitoba, Winnipeg, Manitoba, Canada;* [2]*Departments of Diagnostic Radiology and Physics, c/o Center for Neuroscience Studies, Queen's University, Kingston, Ontario, Canada*

Study design: Functional magnetic resonance imaging (fMRI) of the spinal cord (spinal fMRI) was used to detect neuronal activity elicited by passive and active lower limb movement tasks, in regions caudal to the injury site in volunteers with spinal cord injury.
Objectives: The objectives of this project are: (1) to assess the use of spinal fMRI as a tool for detecting neuronal function in the spinal cord below an injury, and (2) to characterize the neuronal response to active and passive movement tasks.
Setting: Institute for Biodiagnostics, National Research Council of Canada, Winnipeg, Manitoba, Canada.
Methods: fMRI of the spinal cord was carried out in 12 volunteers with cervical or thoracic spinal cord injuries. Spinal fMRI was carried out in a 1.5 T clinical MR system using established methods. Active and passive lower limb movement tasks were performed, and sagittal images spanning the entire lumbar spinal cord were obtained.
Results: Activity was detected in all volunteers regardless of the extent of injury. During both active and passive participation, activity was seen caudal to the injury site, although the number of active voxels detected with passive movement was less than with the active movement task. Average percent signal change was 13.6% during active participation and 15.0% during passive participation.
Conclusions: Spinal fMRI is able to detect a neuronal response during both active and passive lower limb movement tasks in the spinal cord caudal to the injury site.
Sponsorship: This work was funded by a grant from the International Spinal Research Trust (UK) and the Canada Research Chairs Program.
Spinal Cord (2007) **45,** 485–490; doi:10.1038/sj.sc.3102019; published online 23 January 2007

Keywords: injury; MRI; fMRI; human; magnetic resonance

Introduction

Spinal cord repair strategies for recovery of function are becoming a possibility for those with spinal cord injury.[1] As these repair strategies reach clinical trials, it is important to accurately assess the entire spinal cord so that the response to treatment can be monitored. Whereas self-report techniques are useful in assessing spinal cord function rostral to the injury site, function caudal to the injury is not assessable in this manner. In this study, we investigate the value of functional magnetic resonance imaging (fMRI) of the spinal cord (spinal fMRI) in its ability to detect neuronal function caudal to the injury site during specific lower limb movement tasks.

fMRI of the spinal cord is a noninvasive tool that can be used to investigate neuronal activity and reveal important information regarding spinal cord function.[2,3] Spinal fMRI reveals neuronal function indirectly via changes in blood flow and blood oxygen levels that occur near metabolically active gray matter.[4] The signal change arises in part from the blood oxygen level-dependant (BOLD) contrast, which is based on the metabolic activity that occurs in active neuronal tissues. When neuronal firing rates increase, nerve cell bodies take up more oxygen. To compensate, an excess increase in blood supply to neurons occurs resulting in a decrease in the concentration of deoxygenated hemoglobin. Deoxygenated hemoglobin in blood acts as a magnetic

*Correspondence: PW Stroman, Departments of Diagnostic Radiology and Physics, c/o Center for Neuroscience Studies, 231 Botterell Hall, Queen's University, Kingston, Ontario, Canada K7L 2V7

Statement on ethics: We certify that all applicable institutional and governmental regulations concerning the ethical use of human volunteers were followed during the course of this research resonance (MR) contrast agent that causes the MR signal to decay more quickly. At the time of recording, the MRI signal is stronger from metabolically active areas because the signal has not decayed as quickly as in the surrounding nonactive tissues. However, an equally important contrast mechanism in spinal fMRI is caused by signal enhancement from extravascular water protons (SEEP) which results from an increase in water content in the active neural tissues.[5,6] This mechanism is hypothesized to be related to swelling of neurons and/or glial cells that has been shown to arise at sites of neuronal activity.[7–9] In addition, the increased blood flow to the active tissues has been shown to be accompanied by increased intravascular pressure, and increased production of extracellular fluid at sites of neuronal activity.[10,11] The net effect is a local increase in water content near active neural tissues which causes a higher MR signal intensity. Thus, spinal fMRI indirectly detects neuronal activity in spinal cord gray matter, and allows for mapping of spinal cord responses to sensory and motor stimulation. This method has proven useful for detecting neuronal activity in both healthy and injured spinal cords in response to innocuous and noxious thermal stimulation, in which the distribution of activity and the signal intensity change were found to differ between conditions.[2] The neuronal response in the lumbar spinal cord to participation in active and passive lower limb movement tasks was characterized in a previous investigation involving healthy volunteers with spinal fMRI.[12] The current study aims to detect and characterize the neuronal response to these same tasks with spinal cord injured (SCI) volunteers.

Currently, the American Spinal Injury Association[13] (ASIA) assessment scale is the standard for classifying spinal cord injuries. This self-report technique involves a battery of light touch and pin prick examinations to assess where a patient has preserved sensory perception. To determine preserved motor function, the patient's ability to move specific key muscle groups is assessed. Patients are categorized as ASIA A, B, C, or D, indicating the preservation of sensory and/or motor functions below the injury level. ASIA A indicates no sensory or motor function preserved, ASIA B indicates sensory but not motor function is preserved, ASIA C indicates motor function is preserved but in a substantially weakened condition, and ASIA D indicates motor function is preserved in a condition sufficient for near-normal use. Further information regarding the condition of the spinal cord caudal to the level of injury must be obtained by invasive measures or deduced from reflex actions. Electrophysiological techniques such as somatosensory-evoked potential, H-reflex, or stretch reflexes are capable of assessing residual function following a spinal cord injury. The utility of these techniques is limited by the incomplete scope of information that is obtained with each measure, such that a combination of measures is required in order to determine residual function. For example, somatosensory evoked potentials examine conduction along large areas of the body and the results can be affected by peripheral damage, nerve root damage, or spinal cord damage without revealing where along the pathway the damage has occurred. Similarly, an increase in reflexes will suggest that there is damage to the upper motor neurons, but will not reveal the degree of damage, or whether the damage is complete or incomplete. Although these methods are useful for many research applications, they require specific equipment and are time-consuming, and are therefore not in routine clinical use. Thus, should spinal fMRI be successful at detecting neuronal activity below the injury site in SCI patients, this method would be of considerable value for those assessing an injury, planning a treatment strategy, or monitoring recovery of function during and post-treatment. These methods are based on standard clinical MRI systems without special modifications. Analysis can be carried out at a later time on a separate computer using published methods.[2–4] The resulting images reveal a large extent of the spinal cord, which can be displayed in axial, coronal, or sagittal orientation resulting in an effectively 3D display. This noninvasive technique is able to show where functional activity occurs in response to a stimulus regardless of a patient's ability to feel the stimulus, a feature the ASIA assessment scale is lacking. The results can be assessed in relation to those from normal healthy control subjects such that the similarities and differences can be identified. Previous work involving healthy control subjects is available for this comparison.[12]

We hypothesize that neuronal activity is detectable, using spinal fMRI, caudal to an injury site in response to lower limb movement, and that characterization of the neuronal activity in response to passive and active motor tasks in SCI volunteers is possible.

Methods

Participation

Twelve volunteers (11 male, one female) with spinal cord injury participated in this study. The participants' injuries, following the ASIA, were classified as four participants with ASIA A, three with ASIA B, three with ASIA C, and two with ASIA D. The volunteers participated in active and/or passive lower limb movement tasks according to their abilities. During the active task, participants with ASIA C and D moved their feet in an alternating flexion-extension manner. During the passive task, all participants passively allowed their feet to be moved by the experimenter. In both conditions, the movements were kept in time with the noise of the scanner in order for all volunteers' movements to be kept at the same pace. Subjects were supine on a General Electric phased-array spine MRI coil, with their feet secured to a MRI-compatible pedaling device. The device consisted of two pedals with adjustable straps over the toes, and over and below the ankle, which was secured on a base designed to fit the MR bed. The device allowed for rhythmic flexion and extension movements of the ankles and was customized to assist in keeping the movements alternate and equal. Placing a cushion under the knee stabilized the thighs and reduced movement of the upper body thereby minimizing motion effects. The study was reviewed and approved by our Institute's Human Research Ethics Board, and informed consent was obtained from all participants before entering the magnet room.

Imaging
Spinal fMRI was carried out in a block design with two stimulation periods. Images were acquired repeatedly during alternating rest and stimulation periods for a total of 56 time points recorded at 1.5 T in a General Electric Signa Horizon LX clinical MR system. Functional time course data were obtained using single-shot fast spin-echo imaging, as per our established methods[4,12] with sets of eight contiguous 2.8 mm thick sagittal slices. This required a repetition time of 1074 ms per slice, and provided a resolution of $2.8 \times 0.9 \times 0.9\,mm^3$ (R/L, A/P, S/I), with a $12 \times 12\,cm^2$ field of view and a 128×128 matrix. The echo time was set at 37.2 ms. Spatial saturation pulses were applied to eliminate signal from surrounding areas to avoid aliasing and to reduce motion artifacts arising from regions anterior to the spine.

Analysis
Data were analyzed using custom-made analysis programs written in MatLab software (MathWorks, Natick, MA, USA). Before analysis, a line was drawn manually along the anterior edge of the spinal cord in a midline sagittal image taken from the functional data set to indicate the spinal cord position and curvature. The data at each time point of the functional series were combined into a three dimensional volume and linearly interpolated to $0.5 \times 0.5 \times 0.5\,mm^3$ cubic voxels. The volume was resliced transverse to the cord according to the manually drawn reference line, and smoothing was applied only in the rostral-caudal direction. The correlation between a model paradigm and the signal intensity time courses of each voxel was computed to produce a map of correlation *t*-values. A *t*-value threshold of 2.0 ($P = 0.05$) was chosen to identify voxels as active, and these voxels were plotted in color over a gray-scale reference image that was produced by averaging the images obtained at the 56 time points. The activity map was then converted to sagittal, axial, and coronal slices for viewing. The percent signal change and the standard deviation for each task were calculated.

Results

Spinal fMRI was consistently able to detect neuronal activity caudal to the injury site in the lumbar spinal cord of injured volunteers in response to lower limb movement. Evidence of neuronal activity was seen regardless of the level or extent of injury. The number of active voxels that were detected was greater during active participation than during passive participation. Neuronal activity induced by active movement was seen in the dorsal, ventral, and intermediate areas of the spinal cord. During passive movement, neuronal activity was also seen in these areas but there was an overall decrease in the number of active voxels.

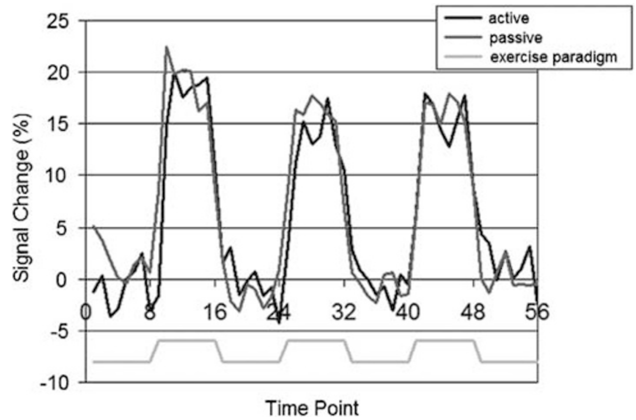

Figure 1 Signal intensity time courses of active regions averaged across participants during active and passive movement follow closely with periods of rest and lower limb movement task, as indicated

The average percent signal change for data acquired during active participation was $13.6 \pm 1.5\%$ and for passive participation was $15.0 \pm 2.8\%$. The alternating periods of rest and movement (movement occurring during time points 9–16, 25–32, and 41–48; see Figure 1) are indicated by a light gray line at the bottom of the graph. The time course of the signal changes fluctuates near baseline during periods of rest and increase dramatically during periods of activity, for both active and passive movement tasks, reflecting the neuronal response to the movement tasks.

As neuronal activity below the injury site was detected in all cases, general characterizations can be made of the distribution. The number of active voxels detected in the spinal cord in this investigation of injured participants was lower than that seen in our earlier study involving healthy individuals as a control group.[12] Of the four volunteers with ASIA A injuries, spinal fMRI demonstrated bilateral dorsal and ventral neuronal responses to the passive task. ASIA B volunteers had results similar in distribution to ASIA A volunteers. In the ASIA C volunteers, activity observed during active participation was predominantly ventral, bilaterally, and during passive participation the activity was mainly in the dorsal areas, with a small ventral contribution. With the ASIA D volunteers, one set of data was discarded owing to motion artifacts caused by the volunteer's spasticity. The remaining ASIA D volunteer's active participation produced predominantly left dorsal activity, in a series in which the volunteer was better able to move the right leg. During passive participation, activity was seen bilaterally in the dorsal and ventral horn with less activity on the right.

In six of the 34 series of data collected, it occurred that movement was generated in one limb only (although the contralateral limb was passively moved

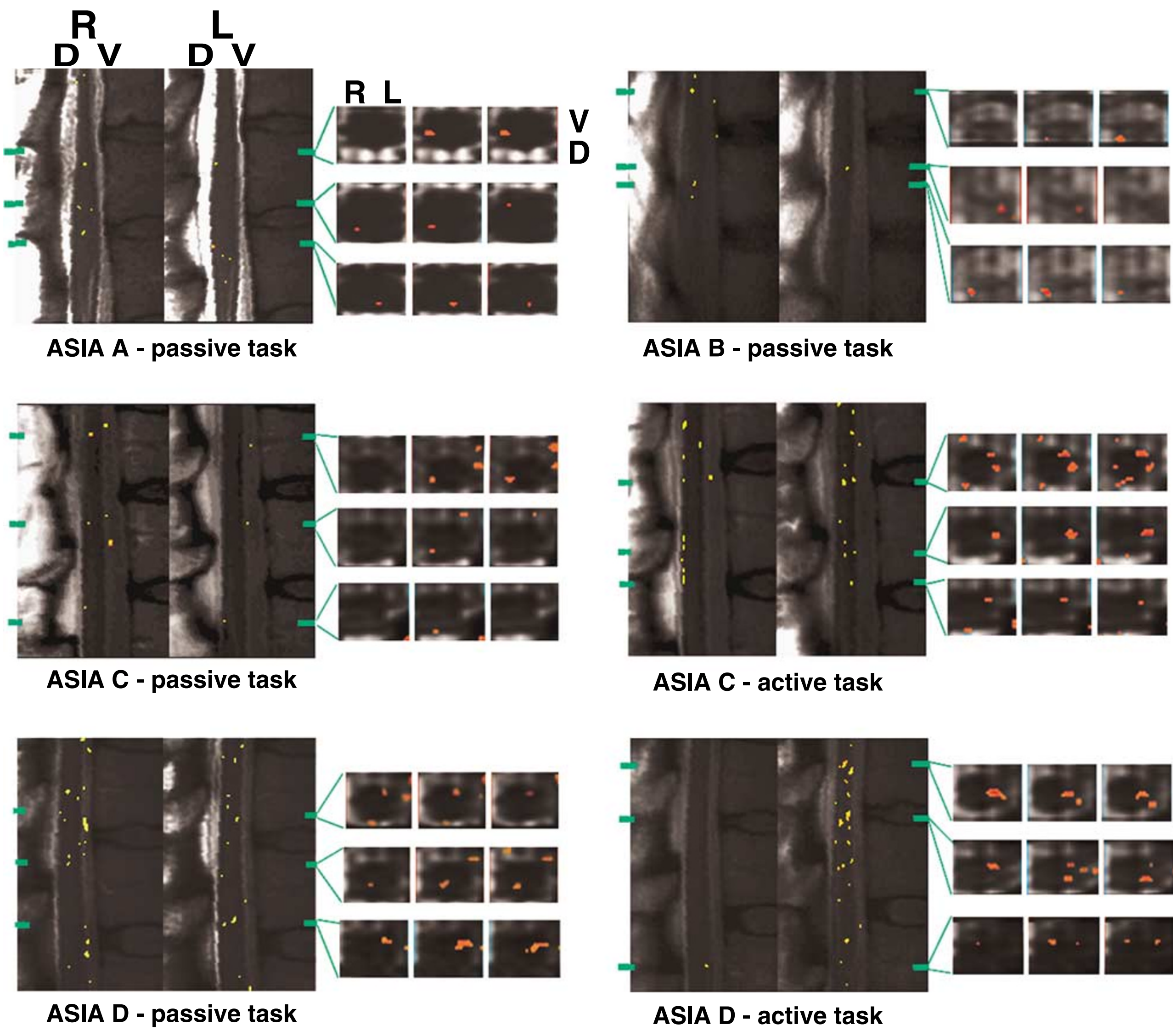

Figure 2 Image orientation: two sagittal slices and nine axial slices from each ASIA classification are shown. Each sagittal slice spans the spinal cord with the lumbar segments approximately mid-image. The slice on the left is taken from the right side of the cord (R) and the slice on the right is taken from the left side of the cord (L). The dorsal aspect is toward the left of the image (D) and the ventral aspect is on the right of the image (V) as indicated by the spinous processes and vertebral bodies, respectively. Sagittal images are oriented with rostral toward the top of each slice, caudal toward the bottom. The axial slices are oriented with the right side of the cord to the left of the image, the left side of the cord to the right of the image, dorsal toward the bottom of the cord, ventral toward the top, rostral to caudal displaying left to right. The level at which the axial slices correspond to the sagittal slices are indicated by green lines. Results from an ASIA A and ASIA B volunteer during passive participation are shown on the top row. Results from an ASIA C volunteer during active and passive participation are shown in the middle row. Results from an ASIA D volunteer during active and passive participation are shown on the bottom row

out of phase by the pedaling device). This unilaterally generated movement corresponded to neuronal activity predominantly on the contralateral side of the spinal cord, in all six cases. For example, one ASIA C volunteer was able to move the right leg only, and neuronal activity was seen bilaterally but with a greater number of active voxels on the left, in the ventral and dorsal horns.

Specific examples from each of the ASIA classification categories are shown and are discussed below. Apparent activity in the CSF is likely the result of CSF flow and/or blood vessels on the surface or on nerve roots. CSF flow and large blood vessels do not occur inside the cord, and so these sources of error do not cause error results within the cord. Figure 2 illustrates the neuronal activity detected in the spinal cord caudal to the injury level in SCI volunteers. Results show activity caudal to the injury site of an ASIA A volunteer during passive participation, on both left and right sides of the cord, in the dorsal and ventral areas. The ASIA B motor complete injury volunteer experienced clonus in the right leg during the passive task condition. Results indicate ventral activity on the left side of the cord, contralateral to the recorded movements, and ipsilateral dorsal activity. Activity is seen in the spinal cord below injury level, although the overall number of active voxels is less than that of healthy volunteers and less than that of the ASIA C and D volunteers.

Neuronal activity in response to an active and passive task, respectively, performed by an individual with an ASIA C injury, is shown in the middle row of Figure 2. Activity appeared to be less in response to the passive task compared to the active task, although similar to that seen in response to the passive task in the ASIA B volunteer. This volunteer was able to move the right foot only, but the left foot continued to be moved passively by the pedaling device. The activity seen in the lumbar cord consists of left and right ventral and dorsal activity, although activity on the left is predominant. During the passive task, activity was seen in the right dorsal and ventral area, and the left dorsal area.

Neuronal activity in response to active and passive movement in an individual with ASIA D motor incomplete injury is shown in the bottom row of Figure 2. This individual was better able to move the right than left foot during the active task, and results show increased activity on the left side of the cord in both the dorsal and ventral areas. During the passive task, the experimenter was able to move both feet equally, and activity is shown evenly distributed on both sides of the cord, and is found in the dorsal and ventral horns.

Discussion

Neuronal activity was detected caudal to the injury site in all volunteers during passive and active movement tasks. The results demonstrate that neuronal activity occurs in the lumbar spinal cord caudal to an injury site, both in complete and incomplete injured volunteers, and that spinal fMRI is capable of detecting this neuronal response. During active participation, neuronal activity is seen bilaterally in the dorsal and ventral horns, indicating a neuronal response to motor and sensory stimulation as would be expected with purposeful movement. During passive participation, some ventral horn activity is seen, possibly indicating reflex activity; however, the bulk of the activity is seen in the dorsal horn, indicating both the dorsal component of the motor terminations as well as the response to sensory stimulation that would be expected with the mechanical and proprioceptive information produced by this type of movement. The main pathway involved in the control of voluntary movement, and therefore recruited during the active movement task, is the corticospinal tract. Within the gray matter, corticospinal axons terminate in lamina IV–VII in the dorsal and ventral horns and in the intermediate gray matter.[14] This broad distribution of termination of corticospinal neurons contributes to the activity seen in both the ventral and dorsal horns and intermediate areas of the spinal cord in response to the voluntary movement during the active task. Further contribution to the activity in the dorsal horns can be accounted for as the tactile sensory pathways also synapse here.[15] The ventral spinothalamic tract conveys light touch and modified forms of tactile sensation such as firm pressure, as would occur during the passive as well as active movement tasks. Proprioceptive feedback, also expected to occur in both passive and active movement tasks, is detected by receptors of the muscle spindles, joints, and tendons. The descending branch of these primary afferents terminate in gray matter to establish connections for spinal reflexes, which contributes to the neuronal activity seen in the dorsal horn accounted for by proprioception.

The pattern of activity observed in this study was in some regards similar to that seen in healthy volunteers, as the distribution of activity is similar during active and passive movement tasks and that the amount of activity (ie number of active voxels detected) is lower with passive movement. Active pedaling with healthy volunteers revealed neuronal activity in the ventral horn and dorsal horn bilaterally with an average percent signal change of $11.9 \pm 1.0\%$. During passive pedaling, the healthy volunteers elicited neuronal activity in the dorsal and intermediate areas bilaterally at lower lumbar segments and in the ventral horn and dorsal horns bilaterally at upper lumbar segments with an average percent signal change of $12.4 \pm 1.1\%$. The magnitude of signal change with the SCI group was $13.6 \pm 1.5\%$ and $15.0 \pm 2.8\%$ during active and passive participation, respectively.

Our results show that in cases where the movement is bilaterally generated, the neuronal activity appears to be distributed across both sides of the cord. However, in the cases where the movement was generated unilaterally, the neuronal activity appears to be prominent on the contralateral side of the cord in the ventral horn. Indeed, this contralateral neuronal activity was observed in all six of the incidences where unilateral movement was observed. The activity detected is in agreement with known physiology and together with previous findings of increased contralateral activation in response to stimulation of the injured spinal cord suggest that spinal fMRI is providing reliable results.[2,16–19] This occurrence and the implications for recovery of function in injured persons have recently come under closer investigation.[19]

Investigation of neuronal circuitry caudal to a spinal cord injury site is required. There are specific difficulties with this clinical population that hinder spinal fMRI progress. The flexor reflex and the crossed extensor reflex are suppressed as a result of activity in descending tracts in healthy people, but can be conspicuous and troublesome for SCI patients owing to lowered threshold.[15] The deficiency in descending inhibitory modulation can result in an excessive amount of neuronal activity in the spinal cord caudal to the injury site, manifest as spasticity. In our study, for example, a volunteer who displayed clonus in response to the stimulation of the lower limb task produced motion artifacts too severe for the data to be analyzed with confidence. Similarly, other limitations of our data in this preliminary study, such as the small number of participants and the heterogeneous SCI sample, prohibit even general conclusions regarding the physiology of specific spinal cord injuries. Before spinal fMRI can be used clinically for individual assessments, a normalization protocol must be developed and implemented. Establishing a normalized coordinate system will provide a reference for comparison across individuals and for interpretation of results. Nevertheless, we were able to successfully achieve the goals of this study by demonstrating that spinal fMRI is capable of detecting neuronal activity caudal to a spinal cord injury and that it is possible to characterize this neuronal response.

Although it is clear from our results that spinal fMRI is capable of detecting differences in neuronal function in response to a stimulus, it is not possible to determine the causes of the neuronal activity observed with the data presented in this study. Conversely, the current ASIA assessment scale lacks sensitivity to neural changes. Supplementing the ASIA assessment with detailed activity maps obtained by means of spinal fMRI may prove to be invaluable for the comprehension of spinal cord physiology and for the design of rehabilitation programs. Investigating neuronal function pre-, during-, and post-rehabilitation programs will provide a quantitative measure of rehabilitation progress. The use of spinal fMRI for such an investigation is shown here to be appropriate. In addition to the benefits of noninvasively detecting neuronal activity caudal to an injury, spinal fMRI can be carried out on any standard clinical MR scanner without requiring special modification.

Conclusions

The results of this study indicate that active voxels demonstrating neuronal activity in the spinal cord in response to both active and passive lower limb movements can be detected using spinal fMRI in individual patients, with adequate sensitivity to reveal areas of neuronal activity in the cord caudal to an injury, with motor and sensory stimulation. This information is expected to lend support to the assessment of spinal cord injury and subsequent rehabilitation. This technique reveals information regarding neuronal function in the spinal cord beyond that which the current ASIA assessment scale is able to provide and is simultaneously less invasive and more informative than electrophysiological methods. This information can be obtained in a noninvasive manner in standard clinical MRI systems, a clear advantage over the existing investigation methods that can be applied caudal to an injury site.

References

1 Borgens R. *Restoring Function to the Injured Human Spinal Cord*. Springer-Verlag, Berlin 2003, pp 138–139.
2 Stroman PW *et al*. Noninvasive assessment of the injured human spinal cord by means of functional magnetic resonance imaging. *Spinal Cord* 2004; **42:** 59–66.
3 Stroman PW, Tomanek B, Krause V, Frankenstein UN, Malisza KL. Mapping of neuronal function in the healthy and injured human spinal cord with spinal fMRI. *NeuroImage* 2002; **17:** 1854–1860.
4 Stroman PW. Magnetic resonance imaging of neuronal function in the spinal cord: spinal FMRI. *Clin Med Res* 2005; **3:** 146–156.
5 Stroman PW, Krause V, Malisza K, Frankenstein U, Tomanek B. Extravascular proton-density changes as a non-BOLD component of contrast in fMRI of the human spinal cord. *Magn Reson Med* 2002; **48:** 122–127.
6 Stroman PW, Kornelsen J, Lawrence J, Malisza KL. Functional magnetic resonance imaging based on SEEP contrast: response function and anatomical specificity. *Magn Reson Imaging* 2005; **23:** 843–850.
7 Sykova E, Vargova L, Prokopova S, Simonova Z. Glial swelling and astrogliosis produce diffusion barriers in the rat spinal cord. *Glia* 1999; **25:** 56–70.
8 Svoboda J, Sykova E. Extracellular space volume changes in the rat spinal cord produced by nerve stimulation and peripheral injury. *Brain Res* 1991; **560:** 216–224.
9 Sykova E. Modulation of spinal cord transmission by changes in extracellular K+ activity and extracellular volume. *Can J Physiol Pharmacol* 1987; **65:** 1058–1066.
10 Fujita H, Meyer E, Reutens DC, Kuwabara H, Evans AC, Gjedde A. Cerebral [15O] water clearance in humans determined by positron emission tomography: II. Vascular responses to vibrotactile stimulation. *J Cereb Blood Flow Metab* 1997; **17:** 73–79.
11 Ohta S, Meyer E, Fujita H, Reutens DC, Evans A, Gjedde A. Cerebral [15O]water clearance in humans determined by PET: I. Theory and normal values. *J Cereb Blood Flow Metab* 1996; **16:** 765–780.
12 Kornelsen J, Stroman PW. FMRI of the lumbar spinal cord during a lower limb motor task. *Magn Reson Med* 2004; **52:** 411–414.
13 American Spinal Injury Association. *International Standards for Neurological Classification of Spinal Cord Injury* (revised, 2002). American Spinal Injury Association: Chicago, IL 2002.
14 Lin VW. *Spinal Cord Medicine: Principles and Practice*. Demos Medical Publishing Inc.: New York 2003.
15 Kiernan JA. *Barr's The Human Nervous System an Anatomical Viewpoint*. 7th edn. Lippincott-Raven Publishers: Philadelphia, 1998, pp 348–349.
16 Kawashima N, Nozaki D, Abe M O, Akai M, Nakazawa K. Alternate leg movement amplifies locomotor-like muscle activity in spinal cord injured persons. *J Neurophysiol* 2005; **93:** 777–785.
17 Kautz SA, Patten C, Neptune RR. Does unilateral pedaling activate a rhythmic locomotor pattern in the non-pedaling leg in post-stroke hemiparesis? *J Neurophysiol* 2006; **95:** 3154–3163.
18 Ferris DP, Gordon KE, Beres-Jones JA, Harkema SJ. Muscle activation during unilateral stepping occurs in the nonstepping limb of humans with clinically complete spinal cord injury. *Spinal Cord* 2004; **42:** 14–23.
19 Jankowska E, Edgley SA. How can corticospinal tract neurons contribute to ipsilateral movements? A questions with implications for recovery of motor functions. *Neuroscientist* 2006; **12:** 67–79.