NeuroImage xxx (2010) xxx–xxx

Contents lists available at ScienceDirect

NeuroImage

journal homepage: www.elsevier.com/locate/ynimg

A quantitative comparison of BOLD fMRI responses to noxious and innocuous stimuli in the human spinal cord

Paul E. Summers [a], Diana Ferraro [a], Davide Duzzi [a], Fausta Lui [a], Gian Domenico Iannetti [b,c], Carlo A. Porro [a,*]

[a] Dipartimento di Scienze Biomediche, Univ. Modena e Reggio Emilia, via Campi 287, I-41100 Modena, Italy
[b] Department of Neuroscience, Physiology and Pharmacology, University College London, London, UK
[c] Department of Physiology, Anatomy and Genetics, University of Oxford, Oxford, UK

ARTICLE INFO

Article history:
Received 15 September 2009
Revised 7 December 2009
Accepted 13 January 2010
Available online xxxx

ABSTRACT

Recent studies have shown that functional magnetic resonance imaging (fMRI) can non-invasively assess spinal cord activity. Yet, a quantitative description of nociceptive and non-nociceptive responses in the human spinal cord, compared with random signal fluctuations in resting state data, is still lacking. Here we have investigated the intensity and spatial extent of blood oxygenation level dependent (BOLD) fMRI responses in the cervical spinal cord of healthy volunteers, elicited by stimulation of the hand dorsum (C6–C7 dermatomes). In a block design fMRI paradigm, periods (20 s each) of repetitive noxious (laser heat) or innocuous (brushing) stimulation were alternated with rest. To estimate the level of false positive responses, functional images were acquired during a separate run while subjects were at rest. In a first analysis of averaged peristimulus signals from all voxels within each half of the spinal cord, we found bilateral fMRI responses to both stimuli. These responses were significantly larger during noxious than during innocuous stimulation. No significant fMRI signal change was evident over corresponding time periods during the Rest run. In a second, general linear model analysis, we identified a voxel population preferentially responding to noxious stimulation, which extended rostro-caudally over the length (4 cm) of the explored spinal cord region. By contrast, we found no evidence of voxel populations responding uniquely to innocuous stimuli, or showing decreased activity following either kind of somatosensory stimulus. These results provide the first false-positive-controlled comparison of spinal BOLD fMRI responses to noxious and innocuous stimuli in humans, confirming and extending physiological information obtained in other species.

Introduction

Anatomical, electrophysiological, and functional imaging studies in experimental animals have provided detailed information on the spinal somatosensory system (Willis and Coggeshall, 2005). Peripheral afferent fibers that carry nociceptive or non-nociceptive information terminate in the ipsilateral dorsal (posterior) horn of the spinal cord, where consistent stimulus-induced increases in metabolic activity have been detected using autoradiographic techniques (Kadekaro et al., 1985; Coghill et al., 1991; Porro et al., 1991). Peripheral nociceptive stimulation has been shown to induce more intense and widespread spinal cord responses, also extending to the contralateral side, than innocuous cutaneous stimulation; these bilateral metabolic responses are likely related to activity in reflex arcs, intraspinal and projection systems (Coghill et al., 1991, 1993; Porro and Cavazzuti, 1993; Coghill and Morrow, 2000; Lilja et al., 2006).

Non-invasive characterization of somatosensory activity in the human spinal cord is important not only for relating human data to those obtained from animal models, but also for providing a framework for investigating spinal cord function in pathological states (Kornelsen and Mackey, 2007), e.g., in multiple sclerosis, spinal cord injuries, and chronic pain. Indeed, pain is an important clinical and social problem (Taylor, 2006; McBeth and Jones, 2007; Smith et al., 2007), often associated with plastic modifications of the spinal cord neural circuitry (Scholz and Woolf, 2007; Cervero, 2009).

The spatial and intensity patterns of somatosensory-evoked activity in the human spinal cord, however, are only beginning to be thoroughly investigated with fMRI techniques. This is due largely to the several technical challenges involved with performing fMRI of the spinal cord (Giove et al., 2004; Stroman, 2005; Brooks et al., 2008). Contact thermal stimuli applied to the hand, forearm, or lower leg have been shown to induce fMRI signal increases in sites consistent with the known functional neuroanatomy of the spinal cord, using either spin-echo (Stroman et al., 2002, 2004, 2005) or gradient-echo (Brooks et al., 2008) MR sequences. Intriguingly, bilateral spinal responses have also been described following unilateral proprioceptive (Kornelsen and Stroman, 2004) and tactile (Agosta et al., 2008,

* Corresponding author. Fax: +39 059 2055363.
E-mail address: porro@unimore.it (C.A. Porro).

1053-8119/$ – see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.neuroimage.2010.01.043

2009) stimulations. However, so far no fMRI study has quantitatively compared nociceptive and non-nociceptive activities with random signal fluctuations in the spinal cord of healthy volunteers.

Recently, we have demonstrated side- and rate-dependent fMRI signal increases in the cervical enlargement of the spinal cord during voluntary movement using single-shot, BOLD-sensitive echo-planar imaging (EPI; Maieron et al., 2007). However, single-shot EPI sequences with good BOLD sensitivity are particularly prone to distortions and signal losses induced by fixed magnetic field inhomogeneities in the spinal cord. To reduce these problems, in the present study we have used a multi-shot gradient-echo EPI sequence (Bouwman et al., 2008) with echo time optimized for a 3-Tesla MR scanner.

Here, we aimed to investigate the intensity and the spatial extent of fMRI signal changes in the human cervical spinal cord during noxious (laser heat) and innocuous (brushing) stimulations of the dorsum of the left hand. Specifically, we aimed to test the following points: (1) whether the activity induced by noxious stimuli was greater than the activity elicited by innocuous stimuli; a larger fMRI response to noxious stimuli may be anticipated on the basis (i) of the higher discharge rates of wide dynamic range neurons in response to noxious than to innocuous stimuli (Price and Dubner, 1977; Willis and Coggeshall, 2005), which are indicative of higher levels of afferent synaptic activity, and (ii) of the higher glucose metabolic rates recorded in the rat spinal cord following noxious than innocuous stimuli (Coghill et al., 1993); (2) whether the response in half of the spinal cord ipsilateral to the stimulated side was larger than the response in the contralateral half of the spinal cord; (3) whether separate populations of spinal cord voxels responding preferentially to noxious or innocuous stimuli could be identified. Because a primary concern in spinal fMRI studies is the degree to which physiological noise may introduce artifactual signal variations that could appear as false positive activations, we also collected fMRI data at rest and compared signal changes over time in the presence or absence of somatosensory stimulation.

Materials and methods

Participants

Eleven healthy, right-handed volunteers (five males and six females), aged 20–34 years (mean 24 ± 5) participated in this study. None of them had a known history of neurological or psychiatric illness, or was under medication. All participants gave their written informed consent. All the experimental procedures conformed to the standards set by the Declaration of Helsinki and were approved by the local Committee on Ethics.

Somatosensory stimuli

Brief radiant heat pulses, generated by an infrared neodymium: yttrium–aluminium–perovskite (Nd:YAP) laser with a wavelength of 1.34 μm (Electronical Engineering, Florence, Italy: www.elengroup.com), were used as noxious somatosensory stimuli. At this short wavelength, the skin is very transparent to the laser radiation, and consequently, the laser pulses directly activate Aδ- and C-fiber nociceptive terminals located in the superficial layers of the skin (Iannetti et al., 2006). The infrared laser pulses were directed at the dorsum of the left hand, and a He–Ne laser pointed to the area to be stimulated. The laser beam was transmitted through an optical fiber and its diameter was set at approximately 8 mm (stimulated area, ~50 mm^2). The duration of the laser pulses was 4 ms. The energy of the laser pulse was adjusted, in a preliminary psychophysical session, in order to achieve a verbal rating of 3–4 out of 10 on a numerical rating scale ranging from 0 to 10, where 0 was defined as "no pricking sensation" and 10 was defined as "the most intense pricking sensation imaginable". The average laser energy was 3.5 J (range 3–4 J). To avoid nociceptor fatigue or sensitization, the laser beam was moved randomly after each stimulus over a 2×4 cm rectangular area in the lateral part of the hand dorsum, approximately corresponding to the C6 and C7 dermatomes (Fig. 1A). This is an unavoidable procedure when delivering thermal laser stimuli, and it is commonly performed in all studies recording behavioral, EEG, and fMRI responses to laser stimuli, both in basic research and clinical practice (see guidelines provided in Treede et al., 2003).

Heat pulses were delivered with a mean frequency of 0.45 Hz (interstimulus interval: 2.2 ± 0.1 s). Innocuous somatosensory stimuli consisted of light stroking (200–400 mN) with a soft make-up brush that was moved back and forth continuously over approximately 4 cm in proximal-distal direction, with a frequency of ~2 Hz (i.e., two single strokes per second). These stimuli were applied manually over the same skin area that received the noxious stimulation. None of the subjects perceived the brush stimulus as painful or unpleasant.

Experimental paradigm

The participants were instructed to relax their muscles, direct their attention to the stimulated hand, and not to move in response to the stimuli throughout the fMRI experiments. An experimenter inside the scanner room delivered the stimuli and monitored the absence of hand and forearm movements. Part of the variability in the recorded response (see below) might be due to changes in the volunteers' levels of attention and arousal. However, it is a fairly accepted procedure (e.g., in studies recording laser-evoked potentials) to ask the volunteers to focus their attention on the somatosensory stimulation, without asking for a behavioral feedback (e.g., Iannetti et al., 2006).

Each volunteer underwent two fMRI data collection runs. In the Stimulation run, laser (noxious) and brush (innocuous) stimuli were delivered in separate 20-s blocks, alternated with 20-s blocks of rest. Six laser and six brush stimulation blocks were given in a pseudo-random order. The initial and final rest blocks were prolonged to 40 s: thus, the whole Stimulation run lasted 9 min, during which 135 spinal volumes were acquired (see below). At the end of the Stimulation run, subjects were asked to rate the mean intensity of noxious stimuli on the same verbal scale used in the preliminary psychophysical phase. An fMRI run of the same length (9 min, 135 volumes) but without stimulation (Rest run) was also performed. The Stimulation and Rest runs were separated by a brief interval (approximately 1 min), and their order was balanced between subjects.

fMRI data collection

Functional MRI data were acquired using the neck and two posterior coil elements of a neurovascular coil array, on a 3T scanner (Philips Medical Systems, Best, The Netherlands). A segmented, gradient-echo (GE) echo-planar imaging (EPI) sequence was used (TE: 23 ms, temporal resolution: 4 s, flip angle: 40°), involving four segments (segment TR = 1 s). This sequence has advantages in spatial fidelity compared with conventional single-shot EPI (Backes et al., 2001; Stracke et al., 2005). Fifteen, 4-mm-thick, contiguous para-axial slices were acquired, oriented approximately perpendicular to the longitudinal axis of the spinal cord. The image field of view was 186×140 mm, with acquisition and reconstruction matrices of 170×108 and 190×144, respectively, thus yielding a voxel size of $0.98 \times 0.98 \times 4$ mm. Saturation bands were applied bordering the image volume to prevent aliasing of tissue signals. The stack of slices was centered on the intervertebral disc between the fifth and sixth cervical vertebrae, and covered from the seventh to fourth cervical vertebra. Additional imaging involved the planning and reference scans, anatomical 3D T1-weighted, and both sagittal and axial T2-weighted scans; the latter co-aligned with the functional data.

Please cite this article as: Summers, P.E., et al., A quantitative comparison of BOLD fMRI responses to noxious and innocuous stimuli in the human spinal cord, NeuroImage (2010), doi:10.1016/j.neuroimage.2010.01.043

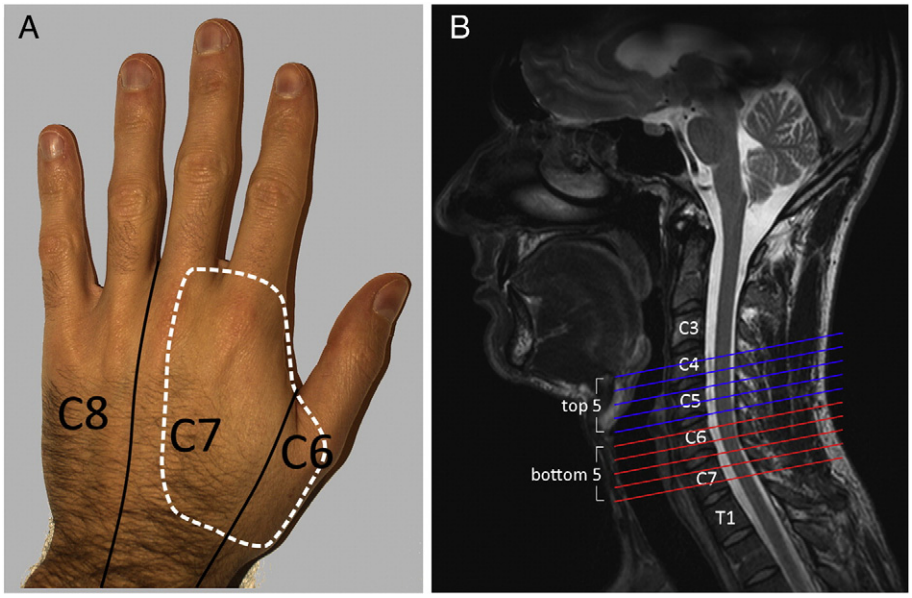

Fig. 1. Stimulation site and MRI slice prescription. Both noxious and innocuous somatosensory stimuli were applied to the dorsum of the left hand (left panel, A), in the region corresponding to the C6 and C7 dermatomes. Functional MRI analysis was conducted on data from 10 consecutive, 4-mm-thick axial slices (in-plane resolution of 0.98 × 0.98 mm), oriented perpendicularly to the longitudinal axis of the spinal cord (right panel, B).

Data related to the cardiac and respiratory activities were recorded, but interference from gradient-induced currents rendered them unusable for subsequent analysis.

fMRI data analysis

Two approaches were taken to analyze the fMRI data. In the first, we compared the stimulus-evoked responses in the fMRI signal averaged over all voxels within each ROI (namely, within each half of the spinal cord), without any a priori voxel selection. In the second, general linear modeling (GLM) was used to identify spinal voxels showing a response matching an expected hemodynamic response function. Preprocessing and analyses of the fMRI data made use of routines from the FSL (www.fmrib.ox.ac.uk/fsl) and AFNI (afni.nimh.nih.gov/afni) software packages. Comparisons of the time profiles and spatial extent of the fMRI responses were performed using a commercial software package for statistical analysis (SPSS, Chicago, IL).

Data preprocessing and ROI definition

Each time series of fMRI volumes (both from the Rest and Stimulation runs) was preprocessed as follows: (1) Images were cropped to a region of approximately 41 × 41 voxels centered on the spinal canal, in order to minimize the impact of structures surrounding the spine on the motion estimation. (2) Motion correction was performed using FLIRT (FSL toolkit) separately for each slice, as sharp discontinuities were observed in the edge contours of some adjacent slices for some subjects. (3) Signals from each image were tested against their time series using 3dTqual (AFNI toolkit), and images differing from the series median by more than 3.5 times the median absolute deviation (MAD) of the series were considered to be outliers and replaced by the mean of the two temporally adjacent scans. (4) The time series were band-pass filtered (0.05–0.5 Hz). No spatial smoothing was applied, because of the small dimensions of the spinal cord and of the expected responses.

There were no systematic differences between the Stimulation and Rest runs in the average absolute values of the estimated motion parameters (x: 0.27 ± 0.04 mm vs. 0.23 ± 0.06 mm; paired $t = 0.472$, $p > 0.6$; y: 0.65 ± 0.13 mm vs. 0.74 ± 0.11 mm; paired $t = -0.848$, $p > 0.4$; r: 0.007 ± 0.001 radians vs. 0.005 ± 0.001 radians; paired $t = 1.471$, $p > 0.15$). Similarly, no significant differences were found between the Stimulation and Rest runs in mean MAD values (0.1267 ± 0.009 vs. 0.1380 ± 0.013; paired $t = -0.802$, $p > 0.4$) or in the number of outlier images (5.57 ± 1.10 vs. 4.47 ± 1.14; $t = -0.865$, $p > 0.4$).

Two regions of interest (ROIs), corresponding to the right and left sides of the spinal cord, were manually drawn on each slice and for each subject. The two ROIs were unified, and a single layer mathematical erosion operator was applied within each image plane to eliminate the boundary voxels, most likely to be subject to partial volume effects with the cerebrospinal fluid and large superficial draining veins. The intersection of the eroded ROI with each of the original ROIs was then taken to define the hemicord ROIs used in subsequent analysis.

In both the Stimulation and Rest runs, the temporal variance of the mean ROI signal of the most inferior five slices was two- to three-fold greater than in the superior ten slices. As this high variance would weaken the sensitivity for detecting a stimulus-induced response in these slices (Parrish et al., 2000), the bottom five slices were excluded from further analysis. The data from three subjects were similarly excluded from further processing: one subject was unable to complete the scanning procedure due to progressive sense of discomfort in the scanner; in two other subjects, the temporal variance in the image signal intensity was three and four times higher than the average of the remaining 8 volunteers. The resulting sets of 10 contiguous slices (Fig. 1B) from 8 subjects in Stimulation and Rest runs were analyzed. Together, the left and right hemicord ROIs contained an average of 573 voxels (range: 450–672) in the 10 slices of the Stimulation run and 579 voxels (range: 474–667) in the Rest run.

ROI signal analysis

In the first analysis, the MRI signal was averaged over all voxels of each hemicord ROI; this was done for each time point, slice, and subject.

As non-BOLD signals may be present in these time series, the spatially averaged ROI values from corresponding time points relative to onset of the laser or of the brush stimulation were then averaged over trials to produce the mean peristimulus BOLD response for each hemicord ROI in each slice. Peristimulus time courses were baseline corrected using the average of the three time points preceding the onset of each stimulation block and the third, fourth, and fifth time points after stimulation end. The same periods of time in the Rest run

Please cite this article as: Summers, P.E., et al., A quantitative comparison of BOLD fMRI responses to noxious and innocuous stimuli in the human spinal cord, NeuroImage (2010), doi:10.1016/j.neuroimage.2010.01.043

were similarly used to generate sham peristimulus plots representing the null condition. Peristimulus plots were compared by analysis of variance (ANOVA), using "Condition" (Laser, Brush, or Rest), "Side" (Ipsilateral or Contralateral to stimulated side), and "Time" as within-subjects experimental factors.

General linear model (GLM) analysis

A GLM analysis was performed using FEAT (FSL toolkit), to identify the spatial distributions and temporal characteristics of specific stimulus-evoked responses within the ROIs. We assumed a gamma-variate hemodynamic response function (mean lag: 6 s; full-width at half-height: 6 s) based on prior brain studies (Glover, 1999), though reports have since suggested a longer delay may be better suited for spinal cord BOLD fMRI (Giulietti et al., 2008). The two modeled explanatory variables were the timings of the Laser and Brush stimulation blocks. The in-plane rotation and displacement estimates from motion correction were included in the model as confounds. Contrasts acting on each single regressor were used to assess the BOLD response associated to each sensory modality. Logical operations were performed on the resulting maps to separate the voxel populations, which were identified by either a single or both contrasts. The same event timings were then applied in a GLM of the Rest data (with its motion estimates as confounds) to estimate the false positive response rate in each subject. An uncorrected value of $p = 0.025$ was defined as the significance threshold in the GLM analysis.

For each obtained contrast, the numbers of identified voxels were calculated as a percentage of all voxels within each ROI and each slice. The spatial extent of activity was compared by ANOVA, with "Condition" (Laser, Brush, or Rest) and "Side" (Ipsilateral or Contralateral to stimulation) as within-subjects experimental factors.

The mean time courses of the identified clusters were used to generate peristimulus plots of signal changes, as described above for the whole ROI analysis. The cluster-specific peristimulus data were compared by a three-way ANOVA, with "Condition", "Side", and "Time" as within-subjects experimental factors.

A value of $p = 0.05$ was defined as the significance threshold in all statistical comparisons of spatial extent and peristimulus time course data.

Results

ROI signal analysis

Somatosensory stimulation induced a significant response in the average fMRI signal extracted from the 10 slices of interest (Time: $F = 7.058$, $p < 0.0001$; Condition × Time interaction: $F = 3.664$; $p < 0.005$). Post-hoc analyses revealed that both noxious (Time: $F = 10.19$, $p < 0.0001$) and innocuous stimuli (Time: $F = 5.03$, $p < 0.02$) elicited a significant increase of fMRI signal over baseline values (Fig. 2). No significant changes over time were found in the Rest run (Time: $F = 0.27$, ns).

In order to get additional information about the spatial distribution of signal changes, we separately averaged the fMRI signals from the left and right halves of the spinal cord, within the 5 superior and 5 inferior slices (Figs. 1B and 3). On the side of the spinal cord *ipsilateral* to the stimulated hand, laser-evoked signal changes in the Stimulation run were (i) significantly greater than signal changes during the corresponding periods in the Rest run, in both the superior and the inferior 5 slices, and (ii) significantly greater than brush-evoked signal changes in the Stimulation run, in the superior slices (Fig. 3). On the side of the cord *contralateral* to the stimulated hand, laser-evoked signal changes in the Stimulation run were significantly greater than signal changes during the corresponding periods in the Rest run in the inferior slices only (Fig. 3).

No significant difference was found between the averaged brush-evoked signal changes in the Stimulation run and signal changes during the corresponding periods in the Rest run.

GLM analysis

In the Stimulation run, voxels responding significantly to laser stimuli were identified in all subjects (Fig. 4). The spatial extent of this "laser" population, averaged across subjects and the 10 slices of interest, was 14.9 ± 3.1% of all ROI voxels (Fig. 5); it was significantly larger than the spatial extent of the corresponding "sham laser" population identified in the Rest run (6.1 ± 1.4%; $F = 10.87$, $p < 0.02$). No significant difference between sides in the spatial extent of the "laser" population was found when either all 10 slices together, or the superior and the inferior 5 slices separately were analyzed.

The analysis of laser-evoked signal changes in the "laser" population showed, as expected, a significant effect of Time ($F = 66.34$, $p < 0.0001$), both for voxels in the ipsilateral ($F = 51.21$, $p < 0.0001$) and in the contralateral ($F = 38.08$, $p < 0.0001$) half of the spinal cord. Increases over baseline values lasted throughout the period of stimulation, and up to 8 s after its end (Fig. 6). No side difference in stimulation-induced signal increases was detected ($F = 0.387$, ns).

In order to test whether the "laser" voxel population indeed uniquely responded to laser stimuli, we analyzed its signal changes during the brush blocks. A significant response to brush stimulation was found (Time: $F = 4.0$, $p < 0.05$), with no side difference ($F = 1.07$, ns); however, the signal increases elicited by noxious stimuli were

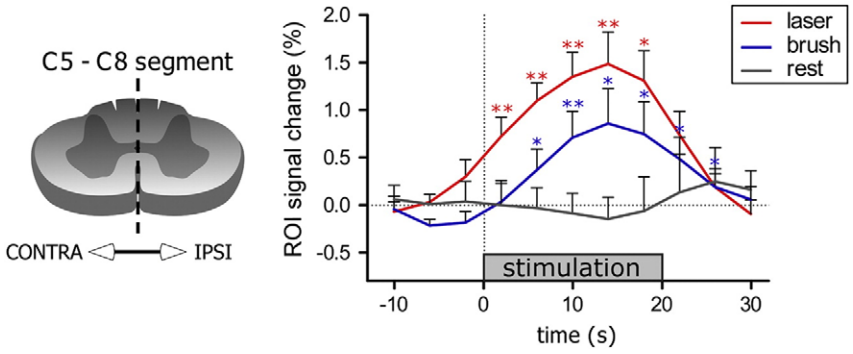

Fig. 2. ROI signal analysis. Graphs display the mean (+S.E.M.) fMRI responses to noxious (laser, in red) and innocuous (brush, in blue) stimulations, averaged over all subjects and slices, in the hemicord ipsilateral to the stimulated hand. The period of stimulation is denoted by the gray bar. The fMRI signal values of the Rest run during the time intervals that would correspond to laser stimulation (rest, in gray) are shown for comparison. Asterisks indicate time points significantly different from baseline, defined as the average of the three time points preceding stimulation; ANOVA + simple contrasts, *$p < 0.05$, **$p < 0.01$. The diagram of the spinal cord on the left side of the figure shows the border between the two ROIs, ipsilateral (ipsi) and contralateral (contra) to the stimulated side, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Please cite this article as: Summers, P.E., et al., A quantitative comparison of BOLD fMRI responses to noxious and innocuous stimuli in the human spinal cord, NeuroImage (2010), doi:10.1016/j.neuroimage.2010.01.043

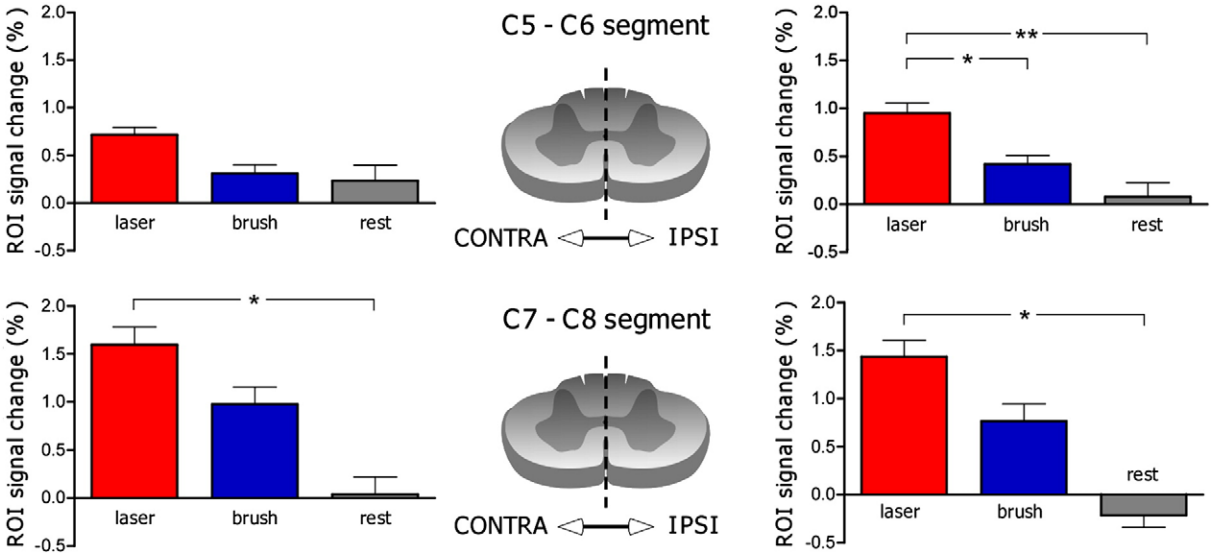

Fig. 3. Mean (±S.E.M.) signal changes from baseline during the laser and brush blocks of the Stimulation run and the corresponding time periods in the Rest run. Asterisks indicate significant differences (*$p<0.05$; **$p<0.01$). "Contra" and "Ipsi" refer to the sides of the cord contralateral and ipsilateral to the stimulated hand, respectively.

significantly higher than those elicited by the innocuous stimuli (average signal increase during the whole stimulation blocks: 2.8% vs. 0.7%; $t=9.784$, $p<0.0001$; Fig. 6). "Sham laser" voxels in the Rest run showed no significant responses during the sham brush blocks (data not shown).

In the Stimulation run, the voxels exhibiting significant decreases in fMRI signals during the laser blocks ("negative laser" population), averaged across subjects, amounted to between 0.3% and 3.28% of the ROI voxels in individual slices. This "negative laser" population was significantly smaller both than the voxel population showing fMRI signal increases during the same blocks ($t=-3.44$, $p<0.02$) and than the "negative laser" voxel population identified in the Rest run ($t=-3.988$, $p<0.005$). No difference between the positive and negative "laser" populations was found in the Rest run, as expected ($t=0.65$, ns).

In both the Stimulation and Rest runs, approximately 8% of all ROI voxels showed activity related to the "brush" blocks, and roughly one third of these (2.7% of all ROI voxels) were identified as responding to both "brush" and "laser" explanatory variables in the GLM analyses. The fractions of the spinal cord represented by these voxel populations were not significantly different between the Stimulation and Rest runs (average from the 2 hemicords: "brush": 8.32±2.24% vs. 8.45±2.08%; $F=0.002$, ns; "brush+laser": 2.65±0.75% vs. 2.41±1.67%; $F=0.24$, ns). Thus, the GLM analysis did not identify a significantly larger number of brush-related voxels in the Stimulation run than the false positive rate.

Discussion

This study provides the first analysis of fMRI activity induced in the human cervical spinal cord by noxious laser and innocuous brushing stimulation of the hand, in comparison with the false positive responses detected in a control rest scan. We observed the following main findings. (1) The fMRI signal increases elicited by noxious stimulation were significantly larger than those elicited by innocuous stimulation. (2) The magnitude and extent of the response to noxious stimulation was above the false positive rate estimated from the rest scans. (3) The fMRI signal increases elicited by noxious stimuli were bilateral and not significantly larger in half of the cord ipsilateral to the stimulated side. (4) There was a spatial overlap between the

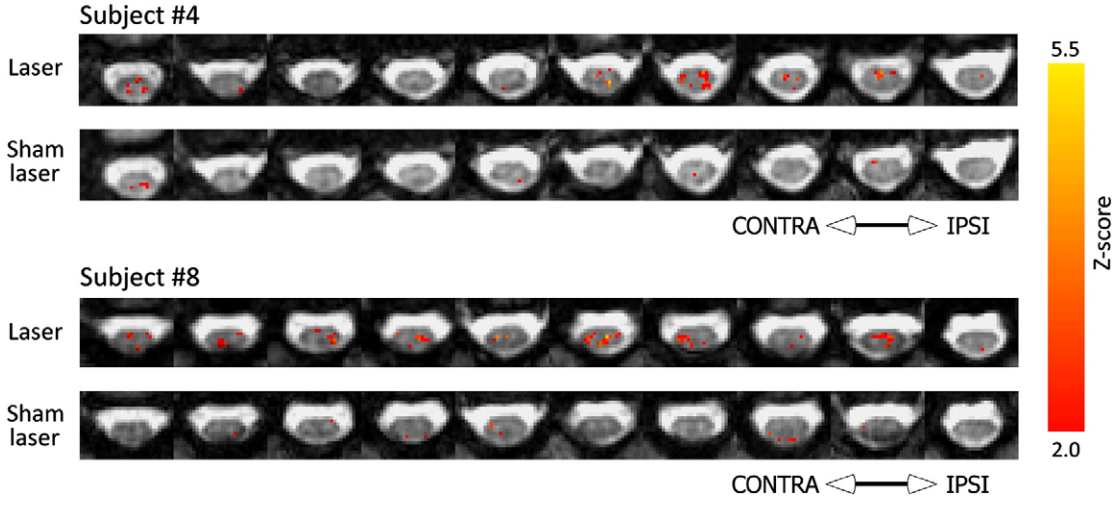

Fig. 4. Representative maps from two subjects, showing the spatial location of voxels preferentially responding to noxious stimulation in the Stimulation run (Laser), or during corresponding time periods in the Rest run (Sham Laser). The most superior slice is displayed to the right of each row. "Contra" and "Ipsi" refer to the sides of the cord contralateral and ipsilateral to the stimulated hand, respectively.

Please cite this article as: Summers, P.E., et al., A quantitative comparison of BOLD fMRI responses to noxious and innocuous stimuli in the human spinal cord, NeuroImage (2010), doi:10.1016/j.neuroimage.2010.01.043

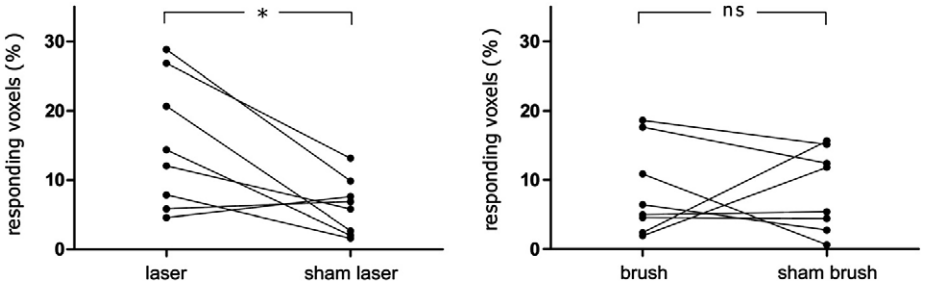

Fig. 5. Spatial extent of the fMRI response to noxious (left graph) and innocuous (right graph) stimulations. Each line represents a subject; *y*-axis represents the number of voxels (expressed as percent of all spinal cord voxels) displaying a significant response. A group-wise significant difference was observed between the Stimulation and Rest runs (actual vs. sham stimulation) for the noxious ($p<0.02$) but not for the innocuous stimuli.

responses elicited by noxious and innocuous stimulations, as expressed by a collective response to brushing among the voxels identified as having a laser response. Further, we could not identify spinal voxels responding uniquely to brush stimuli, or showing decreased activity following either somatosensory stimulation. These results are discussed both with regard to the adopted fMRI protocol and to previous data on spinal cord somatosensory responses.

Methodological considerations

Both ROI and GLM analyses demonstrated significant signal responses to noxious and innocuous stimulations within the Stimulation run—consistent with previous results in humans (Stroman et al., 2002; Stracke et al., 2005; Brooks et al. 2008). We have included in our experimental design a dedicated rest scan, to obtain an estimation of the false positive results. By doing this, we were able to show that the recorded nociceptive-related response was significantly above this false positive level, and thus related to physiological activity. When compared to the Rest run, however, our results do not provide unequivocal evidence for a brush-related response, possibly because this is, as expected, lower than the nociceptive one. Of course, it is well-known that the BOLD fMRI signal cannot be defined "quantitative" in absolute terms, in that it does not measure a single physiological process, and it is expressed in arbitrary units. Thus, the adjective "quantitative" we use in relation to the present findings is not intended in physiological terms, but in terms of spinal BOLD signal changes above noise levels.

In anesthetized rats, GE BOLD fMRI of the spinal cord has been shown to be more sensitive than SE BOLD fMRI (Zhao et al., 2009). Single-shot EPI is widely used in functional magnetic resonance imaging of the brain, but along with being sensitive to BOLD signal changes, it is prone to severe distortion and signal loss artifacts due to magnetic field inhomogeneities. In this study, we have taken several steps to minimize the severity of these artifacts in the collection of the fMRI data. Fat suppression was included in the sequence but is not universally successful due to imperfections in shimming. In the choice of TE, we adopted the water-fat coherence (in-phase) point at 23 ms, adjacent to the optimal TE (21 ms) for spinal cord fMRI at 3T suggested by Bouwman et al. (2008), on the basis of simulations considering the competition of increasing fractional signal change due to BOLD and decreasing available signal due to T2* decay. The use of an in-phase echo time reduces the possibility of local signal deficits due to interference from fat deposits sometimes found in the spinal meninges, or from fat in surrounding tissues, which may be shifted to overlay the spinal cord. In addition, we used voxel dimensions of $1\times1\times4$ mm, thus reducing signal losses due to intravoxel dephasing, while providing a sufficient number of voxels in each spinal hemicord (20–30 in this study) for analyzing the spatial extent of stimulus-evoked activity. The averaging within each spinal level that forms the effective signal for the ROI analysis provides a higher signal-to-noise ratio (SNR) than is present at the voxel level. We note, however, that the voxels acquired here are smaller, and in the absence of spatial averaging, the SNR will be lower than in most brain studies, possibly reducing our sensitivity in GLM analysis.

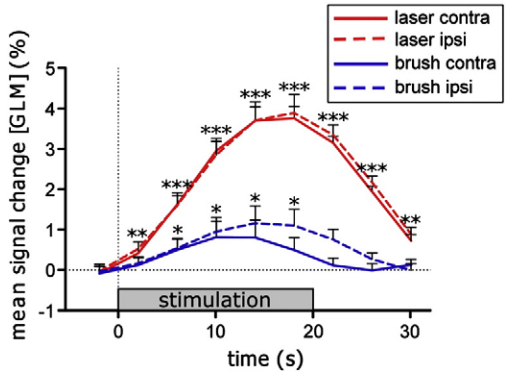

Fig. 6. GLM analysis. fMRI responses to noxious (laser, in red) and innocuous (brush, in blue) stimulations, averaged over all subjects and slices, in voxels preferentially responding to laser stimuli. Asterisks indicate significant differences from baseline (*$p<0.05$, **$p<0.005$, ***$p<0.001$). Data are from the ipsilateral (full lines) and the contralateral (dashed lines) halves of the spinal cord. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

In order to reduce the echo train length, the imaging gradient strengths were maximized, and the field of view was minimized to the limits that they did not pronouncedly reduce SNR. Finally, we adopted a multi-shot EPI acquisition to reduce the echo train length, as this has been shown to reduce the distortion in spinal EPI images. The temporal resolution of the resulting fMRI time series is consequently dependent on the number of shots (four in this study) and, in general, is lower than that of a single-shot scan.

Motion, respiration, and blood pulsation constitute serious confounds in spinal fMRI studies. A recognized complication of multi-shot EPI of the spinal cord is that motion between shots can lead to ghosting, as was detected in up to 5% of images in each run acquired for this study. In respect to the use of multi-shot EPI for spinal fMRI, the possible detrimental effect of its motion sensitivity remains to be fully weighed against its greater immunity from distortion and spatial variation in BOLD sensitivity relative to single-shot EPI. It is not clear whether more sophisticated methods of using physiological data to reduce the risk of detecting false positive activations in single-shot EPI data (Glover et al., 2000; Stroman, 2006) can be directly applied to multi-shot EPI, as adjacent lines near the center of *k*-space are traversed at distinct points in the cardiac and respiratory cycles; therefore, the assignment of an image to unique phases in these cycles is not straightforward. It may be possible to apply autofocus techniques to the *k*-space data in order to reduce the severity of the artifacts, while strategies that weight the time-series data to minimize the influence of corrupted images may improve the analysis without interfering with the image data directly.

Please cite this article as: Summers, P.E., et al., A quantitative comparison of BOLD fMRI responses to noxious and innocuous stimuli in the human spinal cord, NeuroImage (2010), doi:10.1016/j.neuroimage.2010.01.043

Spinal activity related to noxious and innocuous cutaneous stimulations

In the present study we chose innocuous tactile and noxious laser stimuli, which are known to selectively activate Aβ mechanoreceptive fibers, and Aδ and C nociceptive fibers, respectively (Baumgartner et al., 2002).

In experimental animals, different ascending pathways carrying somatosensory information have been demonstrated (see review in Willis and Coggeshall, 2005), and several of them are also present in humans (Willis and Westlund, 2004). Non-nociceptive information from the body surface reaches higher centers mainly through the dorsal column pathway, ending in the gracile and cuneate nuclei; this pathway predominantly contains ascending branches of primary sensory neurons but also includes post-synaptic fibers originated from second-order neurons in the intermediate laminae of the spinal cord dorsal horn. By contrast, nociceptive information reaches the brain through several routes, namely the spinothalamic, spinoreticular, spinomesencephalic, and other pathways targeting limbic regions (Willis and Westlund, 2004). Cell bodies of these second-order neurons are located in the superficial and deep portions of the dorsal horn but also (especially for the spinoreticular tract) in the anterior horn (laminae VII and VIII). Their axons mainly cross the midline and ascend in the anterolateral white matter on the contralateral side of the spinal cord. Metabolic mapping studies in rats have shown that although increases in the 2-deoxyglucose uptake induced by somatosensory stimuli peak in the spinal cord gray matter, less intense changes are also evident in white matter tracts (Kadekaro et al., 1985; Porro et al., 1991). Because metabolic (Magistretti, 2006) and BOLD fMRI (Logothetis et al., 2001) stimulus-evoked changes are mainly related to synaptic activity, we hypothesize that the increases in fMRI signals we detected here are mainly related to nociceptive information reaching second-order gray matter neurons (either projection neurons or interneurons). The segmentation and erosion procedures applied to the spinal images make it less likely that downstream signal changes in large draining veins at the spinal cord surface (see Zhao et al., 2008; Cohen-Adad et al., 2009) contribute to our results to a significant extent.

On the basis of electrophysiological and functional imaging studies of the spinal cord in rodents, it has been suggested that both the frequency of impulse discharges and the total number of activated neurons are critical for encoding noxious events and discriminating their intensity (Coghill et al., 1993). Therefore, it can be hypothesized that acute cutaneous noxious stimulation would be able to elicit more intense and widespread fMRI responses than innocuous stimuli. This hypothesis is supported by recent fMRI studies in anesthetized rats, using different acquisition techniques (Lawrence et al., 2004, 2008; Lilja et al., 2006; Zhao et al., 2008, 2009). Lilja et al. (2006) found that noxious subcutaneous electrical stimulation of a hindlimb induced a graded (intensity-dependent) increase in the magnitude and spatial extent of BOLD fMRI activity in the lumbar spinal cord; fMRI signal increases were also detected in the contralateral intermediate gray matter. Similar increases in activity, extending over several spinal segments, were obtained by Zhao et al. (2008, 2009), employing both BOLD-sensitive and blood volume-weighted fMRI sequences. No significant spinal activation was found in the anesthetized rat during low-intensity electrical stimuli, which selectively activate large diameter A fibers (Lilja et al., 2006; Zhao et al., 2008), and variable responses were found following low-intensity electrical stimulation in anesthetized cats (Cohen-Adad et al., 2009). Another group (Lawrence et al., 2004, 2008) investigated the spinal cord fMRI responses to noxious and innocuous contact heat stimulations of the rat forepaw, using spin-echo imaging. Although the locations of active voxels were similar during both types of stimulation, the magnitude of the response to noxious stimulation was higher. C-*fos* immunohistochemical staining confirmed the presence of neural activity evoked by thermal stimulation (Lawrence et al., 2004).

At the relatively low spatial resolving power of the fMRI technique adopted here, there appears to be at least partial spatial overlap between the response evoked by laser and tactile stimuli. As previously pointed out by Davis (2003), the interpretation of studies comparing the intensity of fMRI signal changes induced by innocuous and noxious stimuli is not straightforward, given the potential contribution of different synaptic terminals and neuronal populations (low threshold, wide-dynamic range and nociceptive specific; Price and Dubner, 1977) to the signal sampled in each single voxel. On the basis of available information from electrophysiological studies in monkeys, higher neuronal activity could be expected in the superficial and deep posterior horn laminae following noxious thermal stimuli, and in the intermediate laminae following innocuous mechanical stimuli (Willis and Coggeshall, 2005). This issue was not addressed here, because the spatial resolution of the employed fMRI technique is insufficient to dissect neural activity arising from these structures.

We observed a considerable rostro-caudal distribution of the spinal fMRI nociceptive responses. This is not unexpected, because (1) the laser beam was moved within two (C6–C7) dermatomes, and (2) the presence of Lissauer's tract is likely to introduce a spread of the rostro-caudal level of the response. Moreover, we found a significant response to noxious stimuli in the contralateral half of the spinal cord; the magnitude of signal increases in the identified voxels is compatible with gray matter activity (Giulietti et al., 2008). These responses might be related to motor reflex activity (Fetz et al., 2000; Willis and Coggeshall, 2005) and descending modulation. It is worth mentioning that, although most nociceptive fibers make synaptic contacts in the ipsilateral side of the cord, some collaterals of Aδ (Light and Perl, 1979) and C fibers (Sugiura et al., 1989) cross the midline to end in the contralateral gray matter. Moreover, contralateral information may be provided by spinal interneurons (Sotgiu et al., 2004) and by spino-supraspinal loops, e.g., those involving the medullary reticular formation (Monconduit et al., 2002).

Our study does not provide evidence for decreased fMRI signals beyond noise levels following either kind of somatosensory stimulation, whereas several previous papers from ours and other groups showed "negative BOLD" responses in several brain areas, which were more intense following noxious than innocuous stimuli (e.g., Iannetti et al., 2005; Moulton et al., 2006; Lui et al., 2008). Although negative BOLD signals have been occasionally found in the rat spinal cord following electrical noxious stimulation, they were most likely to represent false responses, since they were not consistently observed in all animals and acquisition runs (Zhao et al., 2009). Additional studies are required to further characterize this difference between spinal cord and brain fMRI responses.

In conclusion, our findings suggest that physiological responses to nociceptive cutaneous stimuli can be reliably detected using BOLD fMRI in the human cervical spinal cord, and that the functional organization of spinal circuits processing noxious and innocuous information is similar to that found in other species. Further studies are needed to explore in more detail the spatial distribution and extent of the responses to noxious and innocuous stimuli at spinal and supraspinal levels of the somatosensory system.

Acknowledgments

This work was supported by funding from the Human Frontiers of Science Project (Grant team RGP0013/2004), MIUR (PRIN 20073T38Z9/2007, Italy), and Fondazione Istituto Italiano di Tecnologia (BMI Project, IIT, Genova, Italy) to C.A.P. The generous support of the Fondazione Cassa di Risparmio di Modena to the Modena MR Center is gratefully acknowledged. G.D.I. is a University Research Fellow of The Royal Society and acknowledges the support of the Biotechnology and Biological Sciences Research Council (BBSRC).

Please cite this article as: Summers, P.E., et al., A quantitative comparison of BOLD fMRI responses to noxious and innocuous stimuli in the human spinal cord, NeuroImage (2010), doi:10.1016/j.neuroimage.2010.01.043

References

Agosta, F., Valsasina, P., Rocca, M.A., Caputo, D., Sala, S., Judica, E., Stroman, P.W., Filippi, M., 2008. Evidence for enhanced functional activity of cervical cord in relapsing multiple sclerosis. Magn. Reson. Med. 59, 1035–1042.

Agosta, F., Valsasina, P., Caputo, D., Rocca, M.A., Filippi, M., 2009. Tactile-associated fMRI recruitment of the cervical cord in healthy subjects. Hum. Brain Mapp. 30, 340–345.

Backes, W.H., Mess, W.H., Wilmink, J.T., 2001. Functional MR imaging of the cervical spinal cord by use of median nerve stimulation and fist clenching. AJNR Am. J. Neuroradiol. 22, 1854–1859.

Baumgartner, U., Magerl, W., Klein, T., Hopf, H.C., Treede, R.D., 2002. Neurogenic hyperalgesia versus painful hypoalgesia: two distinct mechanisms of neuropathic pain. Pain 96, 141–151.

Bouwman, C.J., Wilmink, J.T., Mess, W.H., Backes, W.H., 2008. Spinal cord functional MRI at 3 T: gradient echo echo-planar imaging versus turbo spin echo. NeuroImage 43, 288–296.

Brooks, J.C., Beckmann, C.F., Miller, K.L., Wise, R.G., Porro, C.A., Tracey, I., Jenkinson, M., 2008. Physiological noise modelling for spinal functional magnetic resonance imaging studies. NeuroImage 39, 680–692.

Cervero, F., 2009. Spinal cord hyperexcitability and its role in pain and hyperalgesia. Exp. Brain Res. 196, 129–137.

Coghill, R.C., Morrow, T.J., 2000. Functional imaging of animal models of pain: high resolution insights into nociceptive processing. In: Casey, K.L., Bushnell, M.C. (Eds.), Pain imaging. IASP Press, Seattle.

Coghill, R.C., Price, D.D., Hayes, R.L., Mayer, D.J., 1991. Spatial distribution of nociceptive processing in the rat spinal cord. J. Neurophysiol. 65, 133–140.

Coghill, R.C., Mayer, D.J., Price, D.D., 1993. The roles of spatial recruitment and discharge frequency in spinal cord coding of pain: a combined electrophysiological and imaging investigation. Pain 53, 295–309.

Cohen-Adad, J., Hoge, R.D., Leblond, H., Xie, G., Beaudoin, G., Song, A.W., Krueger, G., Doyon, J., Benali, H., Rossignol, S., 2009. Investigations on spinal cord fMRI of cats under ketamine. NeuroImage 44, 328–339.

Davis, K.D., 2003. Neurophysiological and anatomical considerations in functional imaging of pain. Pain 105, 1–3.

Fetz, E.E., Perlmutter, S.I., Prut, Y., 2000. Functions of mammalian spinal interneurons during movement. Curr. Opin. Neurobiol. 10, 699–707.

Giove, F., Garreffa, G., Giulietti, G., Mangia, S., Colonnese, C., Maraviglia, B., 2004. Issues about the fMRI of the human spinal cord. Magn. Reson. Imaging 22, 1505–1516.

Giulietti, G., Giove, F., Garreffa, G., Colonnese, C., Mangia, S., Maraviglia, B., 2008. Characterization of the functional response in the human spinal cord: impulse-response function and linearity. NeuroImage 42, 626–634.

Glover, G.H., 1999. Deconvolution of impulse response in event-related BOLD fMRI. NeuroImage 9, 416–429.

Glover, G.H., Li, T.Q., Ress, D., 2000. Image-based method for retrospective correction of physiological motion effects in fMRI: RETROICOR. Magn. Reson. Med. 44, 162–167.

Iannetti, G.D., Zambreanu, L., Wise, R.G., Buchanan, T.J., Huggins, J.P., Smart, T.S., Vennart, W., Tracey, I., 2005. Pharmacological modulation of pain-related brain activity during normal and central sensitization states in humans. Proc. Natl. Acad. Sci. U. S. A. 102, 18195–18200.

Iannetti, G.D., Zambreanu, L., Tracey, I., 2006. Similar nociceptive afferents mediate psychophysical and electrophysiological responses to heat stimulation of glabrous and hairy skin in humans. J. Physiol. 577, 235–248.

Kadekaro, M., Crane, A.M., Sokoloff, L., 1985. Differential effects of electrical stimulation of sciatic nerve on metabolic activity in spinal cord and dorsal root ganglion in the rat. Proc. Natl. Acad. Sci. U. S. A. 82, 6010–6013.

Kornelsen, J., Stroman, P.W., 2004. fMRI of the lumbar spinal cord during a lower limb motor task. Magn. Reson. Med. 52, 411–414.

Kornelsen, J., Mackey, S., 2007. Potential clinical applications for spinal functional MRI. Curr. Pain Headache Rep. 11, 165–170.

Lawrence, J., Stroman, P.W., Bascaramurty, S., Jordan, L.M., Malisza, K.L., 2004. Correlation of functional activation in the rat spinal cord with neuronal activation detected by immunohistochemistry. NeuroImage 22, 1802–1807.

Lawrence, J., Stroman, P.W., Malisza, K.L., 2008. Functional MRI of the cervical spinal cord during noxious and innocuous thermal stimulation in the alpha-chloralose- and halothane-anesthetized rat. Magn Reson. Imaging 26, 1–10.

Light, A.R., Perl, E.R., 1979. Spinal termination of functionally identified primary afferent neurons with slowly conducting myelinated fibers. J. Comp. Neurol. 186, 133–150.

Lilja, J., Endo, T., Hofstetter, C., Westman, E., Young, J., Olson, L., Spenger, C., 2006. Blood oxygenation level-dependent visualization of synaptic relay stations of sensory pathways along the neuroaxis in response to graded sensory stimulation of a limb. J. Neurosci. 26, 6330–6336.

Lui, F., Duzzi, D., Corradini, M., Serafini, M., Baraldi, P., Porro, C.A., 2008. Touch or pain? Spatio-temporal patterns of cortical fMRI activity following brief mechanical stimuli. Pain 138, 362–374.

Logothetis, N.K., Pauls, J., Augath, M., Trinath, T., Oeltermann, A., 2001. Neurophysiological investigation of the basis of the fMRI signal. Nature 412, 150–157.

Magistretti, P.J., 2006. Neuron–glia metabolic coupling and plasticity. J. Exp. Biol. 209, 2304–2311.

Maieron, M., Iannetti, G.D., Bodurka, J., Tracey, I., Bandettini, P.A., Porro, C.A., 2007. Functional responses in the human spinal cord during willed motor actions: evidence for side- and rate-dependent activity. J. Neurosci. 27, 4182–4190.

McBeth, J., Jones, K., 2007. Epidemiology of chronic musculoskeletal pain. Best Pract. Res. Clin. Rheumatol. 21, 403–425.

Monconduit, L., Desbois, C., Villanueva, L., 2002. The integrative role of the rat medullary subnucleus reticularis dorsalis in nociception. Eur. J. Neurosci. 16, 937–944.

Moulton, E.A., Keaser, M.L., Gullapalli, R.P., Maitra, R., Greenspan, J.D., 2006. Sex differences in the cerebral BOLD signal response to painful heat stimuli. Am. J. Physiol. Regul. Integr. Comp. Physiol. 291, R257–R267.

Parrish, T.B., Gitelman, D.R., LaBar, K.S., Mesulam, M.M., 2000. Impact of signal-to-noise on functional MRI. Magn Reson. Med. 44, 925–932.

Porro, C.A., Cavazzuti, M., 1993. Spatial and temporal aspects of spinal cord and brainstem activation in the formalin pain model. Prog. Neurobiol. 41, 565–607.

Porro, C.A., Cavazzuti, M., Galetti, A., Sassatelli, L., Barbieri, G.C., 1991. Functional activity mapping of the rat spinal cord during formalin-induced noxious stimulation. Neuroscience 41, 655–665.

Price, D.D., Dubner, R., 1977. Neurons that subserve the sensory-discriminative aspects of pain. Pain 3, 307–338.

Scholz, J., Woolf, C.J., 2007. The neuropathic pain triad: neurons, immune cells and glia. Nat. Neurosci. 10, 1361–1368.

Smith, B.H., Macfarlane, G.J., Torrance, N., 2007. Epidemiology of chronic pain, from the laboratory to the bus stop: time to add understanding of biological mechanisms to the study of risk factors in population-based research? Pain 127, 5–10.

Sotgiu, M.L., Brambilla, M., Valente, M., Biella, G.E., 2004. Contralateral input modulates the excitability of dorsal horn neurons involved in noxious signal processes. Potential role in neuronal sensitization. Somatosens. Motor Res. 21, 211–215.

Stracke, C.P., Pettersson, L.G., Schoth, F., Moller-Hartmann, W., Krings, T., 2005. Interneuronal systems of the cervical spinal cord assessed with BOLD imaging at 1.5 T. Neuroradiology 47, 127–133.

Stroman, P.W., 2005. Magnetic resonance imaging of neuronal function in the spinal cord: spinal FMRI. Clin. Med. Res. 3, 146–156.

Stroman, P.W., 2006. Discrimination of errors from neuronal activity in functional MRI of the human spinal cord by means of general linear model analysis. Magn. Reson. Med. 56, 452–456.

Stroman, P.W., Krause, V., Malisza, K.L., Frankenstein, U.N., Tomanek, B., 2002. Functional magnetic resonance imaging of the human cervical spinal cord with stimulation of different sensory dermatomes. Magn. Reson. Imaging 20, 1–6.

Stroman, P.W., Kornelsen, J., Bergman, A., Krause, V., Ethans, K., Malisza, K.L., Tomanek, B., 2004. Noninvasive assessment of the injured human spinal cord by means of functional magnetic resonance imaging. Spinal Cord 42, 59–66.

Stroman, P.W., Kornelsen, J., Lawrence, J., 2005. An improved method for spinal functional MRI with large volume coverage of the spinal cord. J. Magn. Reson. Imaging 21, 520–526.

Sugiura, Y., Terui, N., Hosoya, Y., 1989. Difference in distribution of central terminals between visceral and somatic unmyelinated (C) primary afferent fibers. J. Neurophysiol. 62, 834–840.

Taylor, R.S., 2006. Epidemiology of refractory neuropathic pain. Pain Pract. 6, 22–26.

Treede, R.D., Lorenz, J., Baumgärtner, U., 2003. Clinical usefulness of laser-evoked potentials. Neurophysiol. Clin. 33, 303–314.

Willis, W.D., Westlund, K.N., 2004. Pain system. In: Paxinos, G., Mai, J.K. (Eds.), The Human Nervous System. Elsevier, San Diego, pp. 1125–1170.

Willis, W.D., Coggeshall, R.E., 2005. Sensory mechanisms of the spinal cord. Kluwer, New York.

Zhao, F., Williams, M., Meng, X., Welsh, D.C., Coimbra, A., Crown, E.D., Cook, J.J., Urban, M.O., Hargreaves, R., Williams, D.S., 2008. BOLD and blood volume-weighted fMRI of rat lumbar spinal cord during non-noxious and noxious electrical hindpaw stimulation. NeuroImage 40, 133–147.

Zhao, F., Williams, M., Meng, X., Welsh, D.C., Grachev, I.D., Hargreaves, R., Williams, D.S., 2009. Pain fMRI in rat cervical spinal cord: an echo planar imaging evaluation of sensitivity of BOLD and blood volume-weighted fMRI. NeuroImage 44, 349–362.

Please cite this article as: Summers, P.E., et al., A quantitative comparison of BOLD fMRI responses to noxious and innocuous stimuli in the human spinal cord, NeuroImage (2010), doi:10.1016/j.neuroimage.2010.01.043