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Spinal fMRI investigation of human spinal cord function over a range of innocuous thermal sensory stimuli and study-related emotional influences

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Abstract

Functional magnetic resonance imaging (fMRI) of the human spinal cord has revealed important details of activity involved with innocuous sensory stimuli, including the primary input to ipsilateral dorsal gray matter and activity in bilateral ventral gray matter regions. The latter is hypothesized to reflect descending modulation from the brainstem and cortex. Here, the functions corresponding to these areas of activity are investigated by varying the temperature of innocuous thermal stimuli, and the order they are presented, across repeated fMRI experiments in the spinal cord and brainstem. Group results and connectivity analyses reveal that the ipsilateral dorsal gray matter (dGM), the primary site of sensory input, also receives inhibitory input from the rostral ventromedial medulla and the locus coeruleus, two components of the brainstem opiate analgesia system. Ipsilateral ventral gray matter (vGM) receives input from the ipsilateral dGM and inhibitory input from the pontine reticular formation, which is involved with coordination of movements by modulation of ventral horn cells. Contralateral vGM regions appear to receive input from only the ipsilateral dGM in these studies. These results provide an unprecedented view of details of human spinal cord function and descending modulation, and have important implications for assessment of the effects of spinal cord trauma and disease by means of fMRI.
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1. Introduction

Functional magnetic resonance imaging (fMRI) has become an important tool for neuroscience research in humans [1] and now the adaptation of fMRI for use in the spinal cord (spinal fMRI) has the potential to provide a view of neuronal activity in the healthy human spinal cord as never before [2] This method has revealed new information about normal sensory and motor function and its modulation by brainstem and cortical structures in the healthy intact cord, as well as after injury or disease (specifically, multiple sclerosis) [3–7].

Our view of human spinal cord function, whether in able-bodied people, or after spinal cord trauma, is based on information from studies of anesthetized or decerebrate animals, postmortem anatomical studies in humans and animals, tracer studies such as with horseradish peroxidase or from observing the effects of injury and disease [8]. While these studies are essential to our understanding of the anatomy and function of the spinal cord, our knowledge of normal human spinal cord function is based on very little information from normal healthy humans [9–12]. The possibility remains that there are details of the complex networks of neural functions in humans that cannot be observed with these models. Not only may there be significant differences in descending modulation of responses related to affective and cognitive processes between humans and mice, rats and cats, but it is difficult to assess upper extremity function in animal models; there are different arrangements of tracts and possible genetic differences in injury responses [8].

Spinal fMRI has been verified to demonstrate neuronal activity with high sensitivity and reliability, entirely noninvasively, in healthy human volunteers, and therefore provides a potential means for more direct study of normal spinal cord function [3,5–7]. This method is not without its

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limits, however, and the correct interpretation of spinal fMRI results is still being validated. This interpretation must be carried out in reference to the neuronal activity that occurs in the normal healthy human spinal cord, and so, the validation of the interpretation and the study of normal responses must be done in parallel.

A current challenge with spinal fMRI results is the interpretation of activity that is consistently observed in response to innocuous and noxious stimuli, in intermediate or ventral gray matter (GM) regions, including contralateral GM regions [13–15]. As the stimuli in these studies did not produce a reflex motor response to withdraw from the sensation, the primary site of activity is expected to be in the ipsilateral dorsal GM [16]. It is unclear from the literature on spinal cord neuroanatomy how the observed activity relates to innocuous thermal sensations [16]. Is this a previously unknown component of the response, a systematic error in spinal fMRI results or simply an unidentified fMRI representation of a known component of the response? The latter may occur because it is not yet completely known how the activity observed with spinal fMRI compares with the functional neuroanatomy demonstrated by other means such as electrophysiological recordings and tracer studies in animals. There are multiple potential reasons why differences may occur, such as the comparison between spinal cord function between anesthetized or decerebrate animals and healthy humans, and differences in sensitivity between the various methods.

Spinal cord activity in response to a sensory stimulus is the net effect of afferent input to the cord from the periphery and descending modulation from supraspinal structures. Fig. 1 summarizes the areas of activity in the brainstem and spinal cord with normal pain and temperature sensations. Sensory input to the ipsilateral dorsal horn of the spinal cord gray matter is relayed to bilateral ventral motor areas via interneurons. The ventral input depends on the intensity of the thermal stimulus and whether or not it is painful [20–22], in which case it produces the motor reflex to withdraw from the sensation [20]. The responses are modulated, as shown in Fig. 1, by descending input to the dorsal gray matter, ipsilateral to the stimulus, which receives descending input via the raphespinal tract. Intermediate and/or ventral gray matter (GM), receives input via the medullary reticulospinal tract, whereas input to intermediate/ventral GM areas on both sides of the cord is via the pontine reticulospinal tract. The significance of these pathways for the present investigation is that the medullary and pontine reticulospinal tracts are part of the extrapyramidal system and are therefore descending motor tracts which are involved with coordination of movements, by modulation of ventral horn cells. The raphespinal tract originates in raphe nuclei in the pons and medulla, including the nucleus raphe magnus (NRM) and provides descending modulation of nociceptive input to the dorsal horn.

Descending modulation of sensory responses in the spinal cord is known to involve the periaqueductal gray matter (PAG), and the rostral ventromedial medulla (RVM), which includes the NRM and nucleus reticularis paragigantocellularis (PGC) [10,11,23]. The RVM is considered to be the final common output for descending input from the brain [12]. The PAG, RVM and PGC are all part of the brainstem opiate analgesia system which project to the dorsal spinal cord. Electrical and chemical stimulation of some areas of the RVM produce only inhibition of spinal nociceptive processing, some areas produce only facilitation and some areas produce intensity-dependent inhibition or facilitation [12]. Inhibition is predominant and is often tonically active, and descends from the RVM in dorsolateral funiculi, whereas facilitation descends in the ventral/ventrolateral cord, and both are primarily ipsilateral to stimulation and recording sites (that is, in the studies used to trace these areas) [12].

Thus, to summarize, thermal sensory stimulation is expected to produce input to the ipsilateral dorsal horn from sensory afferents and modulatory input from the RVM, which may be inhibitory or facilitory. Ipsilateral ventral regions receive input from the dorsal horn via interneurons, as well as from reticular formation nuclei to modulate the motor response. Similarly, the contralateral ventral gray matter receives input from reticular formation nuclei to modulate motor responses. Other possible contributions to contralateral activity are via the small proportion of axons that descend uncrossed, such as the 10–25% of corticospinal fibres in the anterior and anterolateral tracts. Those in the anterior tract connect bilaterally to lamina VII in the ventral horn, and those in the anterolateral tract connect to dorsal and intermediate regions. There are also interneurons which cross the midline of the cord and may therefore contribute to the contralateral activity observed with spinal fMRI.

For the present study, the hypothesis is that the intermediate/ventral activity that is detected with spinal fMRI in response to innocuous thermal stimulation, is the result of descending modulation from the brainstem, both ipsilateral and contralateral to the site of stimulation. This is investigated by applying a range of innocuous thermal stimuli to the right hands of healthy human volunteers. Spinal fMRI data spanned the cervical spinal cord and the brainstem in order to detect interconnectivity between these regions. The anatomical locations of active regions, and their interactions, were investigated by means of: (1) random-effects group analysis to determine the consistent responses across the 15 healthy participants, (2) partial-least squares (PLS) analysis to determine the significant differences between active regions as a function of stimulus temperature or order of experiments, (3) functional connectivity analysis to determine the consistent relationships between active regions and (4) characterization of signal change responses to determine the specific responses of selected regions to different stimuli. The general linear model (GLM), PLS and functional connectivity methods are independent, and each reveals different properties of the active regions and how they depend on the stimuli that were studied. The combination of these methods is therefore used in an effort to fully characterize the network of responses to innocuous thermal stimuli, in order to

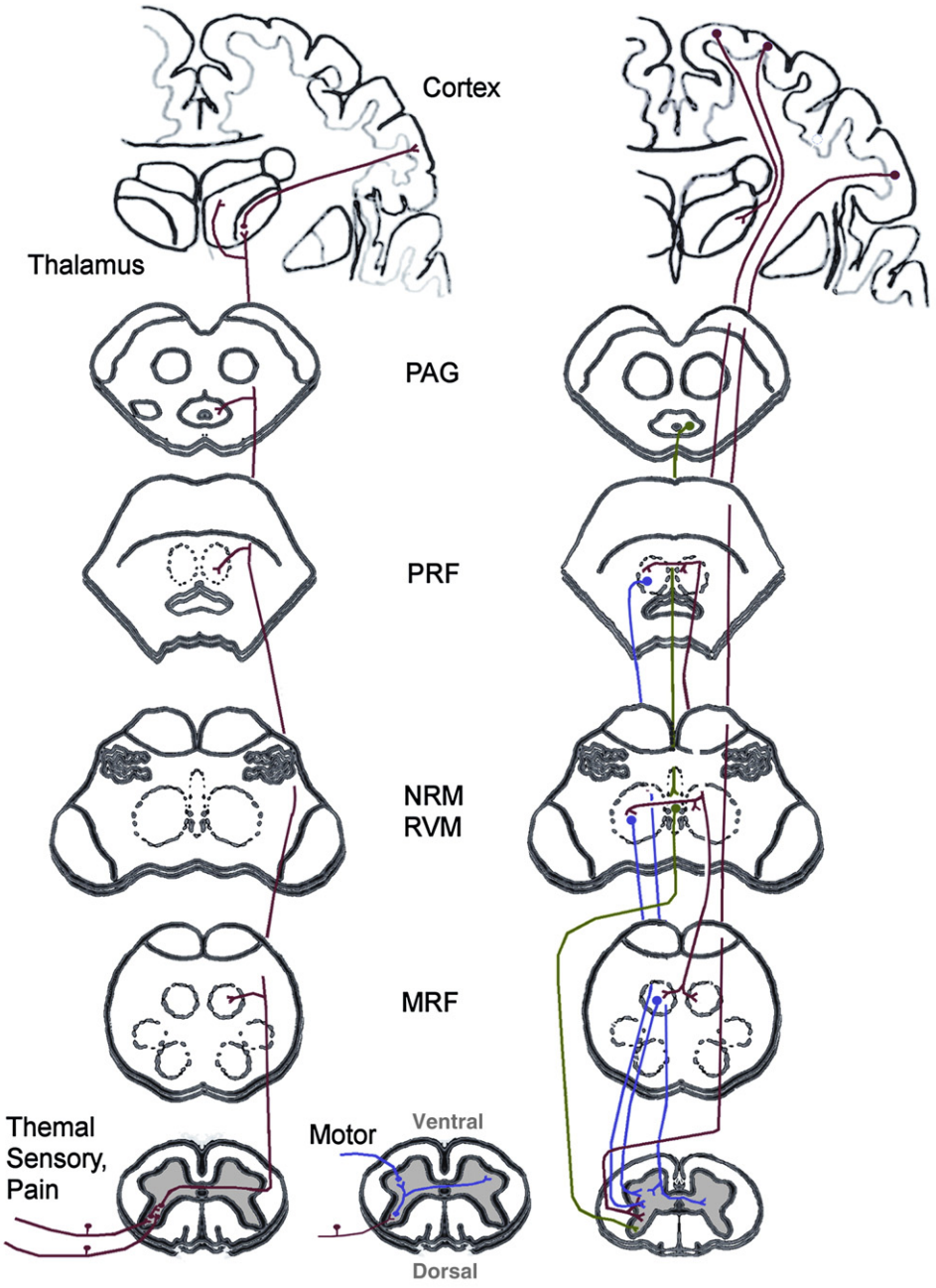

Fig. 1. Summary of ascending (left) and descending (right) pathways and areas of activity involved with temperature and pain sensations applied to the right hand (pathways are drawn according to: [16–19]. The schematic at bottom centre indicates the withdrawal reflex pathways. The spinal cord and brainstem cross-sectional drawings at various levels are in radiological orientation (the right side of the body is to the left in the figure, and dorsal is toward the bottom of the figure) to correspond with the orientation of the fMRI results. The gray matter is also indicated in gray, at the level of the spinal cord. In the left and right diagrams, the red lines indicate spinothalamic (left) and corticospinal (right) pathways; blue lines indicate reticulospinal pathways and green lines indicate the descending analgesia pathways via the PAG and RVM.

determine the neural correlates of the activity detected in each region of spinal cord gray matter.

2. Methods

fMRI of the brainstem and spinal cord was carried out with 15 healthy volunteers (seven males) at 3 T (Siemens Magnetom Trio). The volunteers had no history of central nervous system injury or disease, and all participated after providing informed consent. This study was reviewed and approved by the institutional research ethics board. Volunteers lay supine and entered the magnet head-first. An initial set of localizer images was acquired with a fast gradient-echo sequence in three planes to provide reference information for subsequent slice positioning. Functional image data were

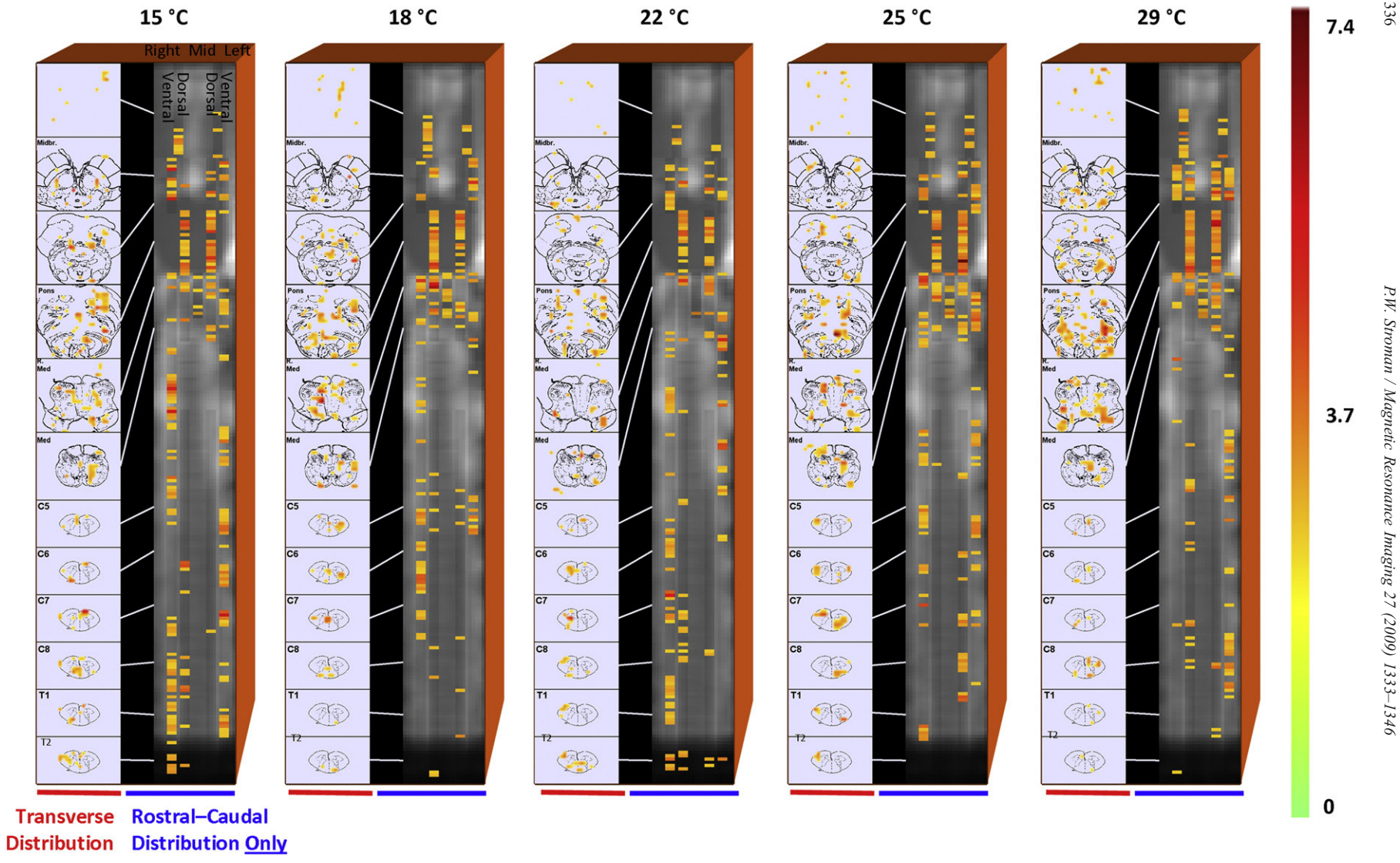

Fig. 2. Combined group results (random-effects analysis) demonstrating consistent areas of activity within the cervical spinal cord and brainstem in 15 healthy volunteers, as a function of temperature of stimulation applied to the thenar eminence on the right hand. The left half of each frame shows the transverse (R/L×A/P) distribution of active regions overlaid onto anatomical drawings in radiological orientation. The activity on each drawing is shown for a rostral-caudal span approximating the corresponding spinal cord segment or region of the brainstem. The right half of each frame shows only the rostral-caudal distribution of activity overlaid onto a spatially normalized mid-line coronal slice, and does not reflect the right-left spatial locations. The R/C activity is represented in columns corresponding to (going from left-to-right) right ventral, right dorsal, midline, left dorsal and left ventral regions. The approximate spatial correspondences between the transverse and R/C representations of the activity distributions are indicated with white lines. The colors represent the significance (T value) of each active voxel across the 15 volunteers, as indicated by the color bar on the right. Only values with a magnitude of T>2.5 are shown.

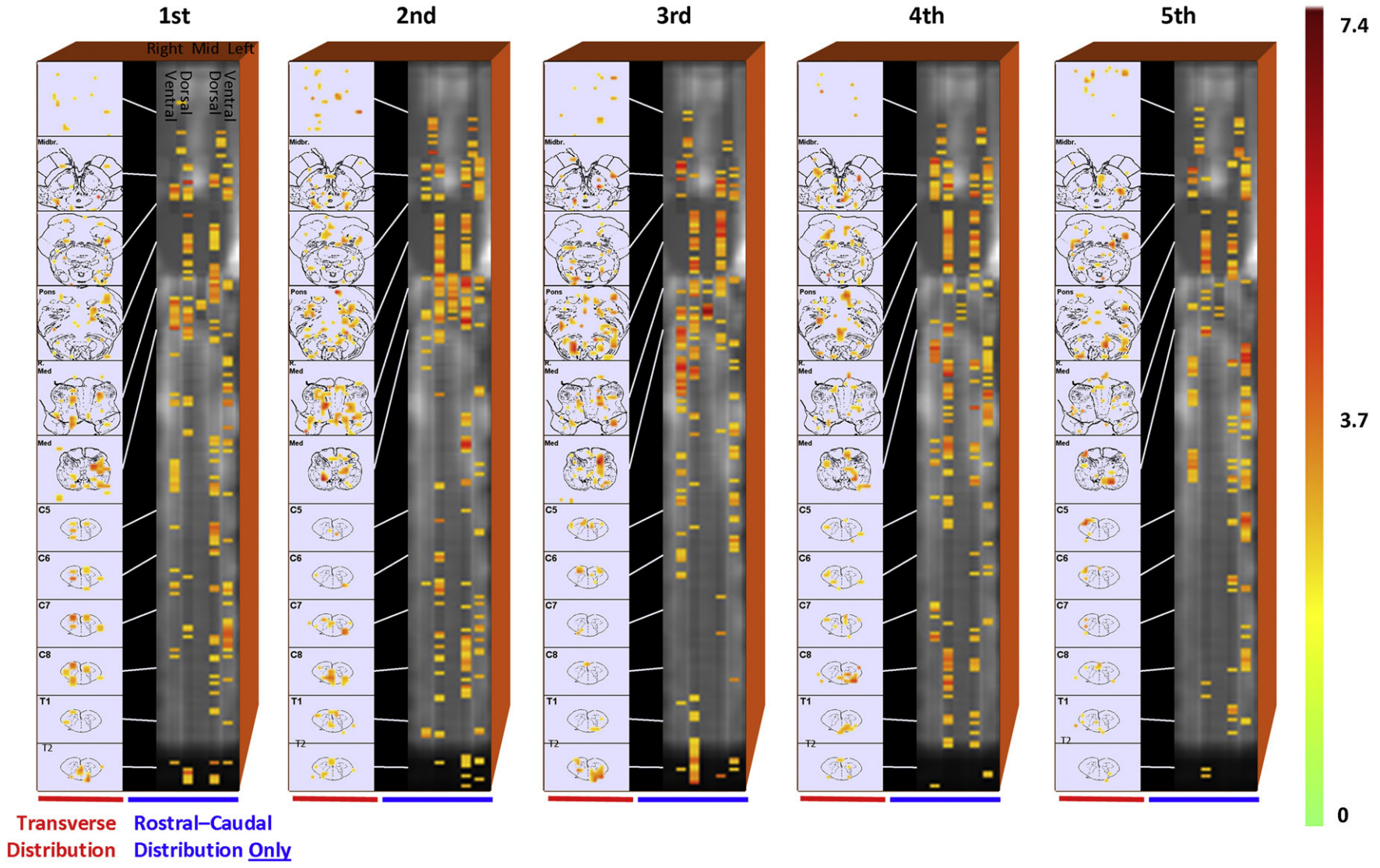

Fig. 3. Combined group results (random-effects analysis) demonstrating consistent areas of activity within the cervical spinal cord and brainstem in 15 healthy volunteers, as a function of the point in the order of repeated experiments, regardless of the stimulus temperature. The image orientation and layout is identical to that used in Fig. 2. The colors represent the significance (T value) of each active voxel across the 15 volunteers, as indicated by the colorbar on the right. Only values with a magnitude of T>2.5 are shown.

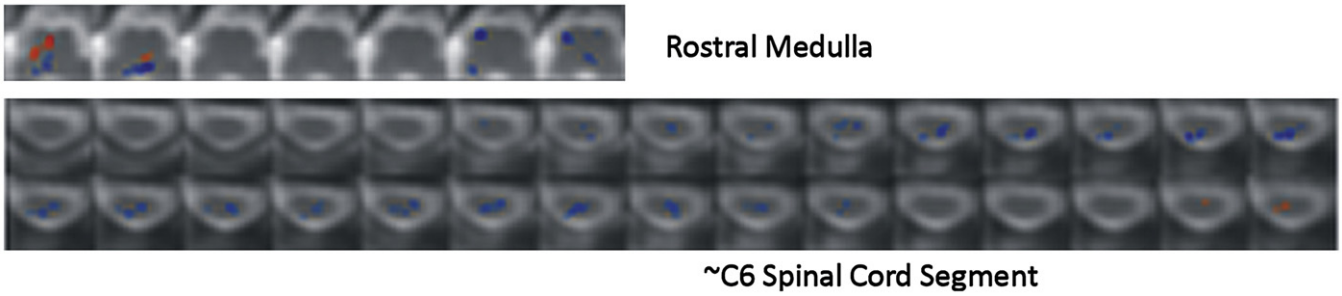

Fig. 4. Partial-least squares (PLS) results, on a voxel-by-voxel basis, showing the contrast between stimulation at lower (15–18°C) and higher (22–25°C) temperatures at selected R/C levels of the spinal cord and brainstem. Levels indicated are in the rostral medulla (first row) and approximately at the sixth cervical (C6) spinal cord segment (second and third rows). Each frame represents a 1 mm thick transverse slice in radiological orientation, shown so that moving 1 frame to the right in the figure is 1 mm more caudal in the anatomy, and the third row is a continuation of the second row. The colors represent the significance (bootstrap ratio >5) with positive values shown in red and negative values in blue. Positive values indicate a higher signal change response with the lower temperatures compared to the higher temperatures, and negative values indicate the opposite occurred.

acquired with a phased-array spine receiver coil, using a half-Fourier, single-shot, fast spin echo sequence (TE=38 ms, effective TR=9 s, 20×10 cm FOV, 192×96 matrix), as described previously [24]. Nine contiguous sagittal slices (2 mm thick) were imaged spanning from above the thalamus to below the C7/T1 intervertebral disc. The resulting voxel size was 1×1×2 mm. Thermal stimulation of the C6 dermatome was produced using a Medoc TSA-II thermal sensory analyzer with the thermal probe placed on the right thenar eminence. Prior to imaging, each volunteer's cold pain threshold was determined, and these fell within a narrow range (±1°C) around 9°C. In each experiment, the stimulator cycled between 32°C for the baseline condition and one of 5 stimulation temperatures: 29°C, 25°C, 22°C, 18°C or 15°C. The lower temperatures therefore caused discomfort but were above the reported pain threshold. Five studies were carried out with each volunteer, one with each of the five stimulation temperatures. The order of the experiments was varied so that every permutation of temperature and place in the order (i.e., first, second, third, etc.) was sampled an equal number of times (three times) across the group of 15 volunteers. Baseline periods were held for 72 s (8 volumes), except for the initial baseline period of 81 s, and stimulation periods were maintained for 45 s (5 volumes). The entire paradigm consisted of three stimulation periods alternated with four baseline periods (48 volumes). During all experiments, the volunteers watched a movie to provide an attention focus.

The resulting 3D image data were analyzed using a GLM, with a basis set consisting of a boxcar model paradigm convolved with the tissue response function [25] and models of cardiac-related motion of the spinal cord as confounds [24,26]. The results demonstrate the required weighting factors, termed "β values," for each element in the basis set to sum to the observed signal intensity time course. The value of β_1 is the magnitude of the pattern matching the stimulation paradigm convolved with the tissue response function, and β_0 is that of the constant function (i.e., the average intensity of the voxel). The individual results are expressed as the significance (T-value) of β_1 for each voxel spanned by the image data. The results were then reformatted and normalized to a consistent coordinate space, defined for the brainstem and spinal cord, to facilitate group comparisons [24]. Combined group results were determined using a random-effects analysis as described by McGonigle et al. [27]. This consisted of calculating the mean and standard deviation of the ratio of β_1/β_0 (represents the relative signal intensity response) across studies to determine the significance of the activity detected in each voxel. Significant activity was assumed for T values of greater than 2.5 or less than −2.5 (corresponds with $P<.01$ for 15 subjects).

Group analyses were also carried out using the PLS method [28] to contrast the signal intensity responses between stimulation at different temperatures and at different times in the order of multiple experiments in each volunteer. This analysis provides temporal and spatial comparisons of responses across all volunteers on a voxel-by-voxel basis and is independent of the GLM analysis described above. The difference between two contrasted responses was concluded to be significant when it was at least five times the sum of the standard deviations of the two responses (i.e., bootstrap ratio ≥5). Details of the PLS method are provided as an Appendix.

An analysis of functional connectivity was also carried out across all voxels of the brainstem and spinal cord, based on correlations between signal intensity responses [29,30]. Again, this is independent of the GLM or PLS analyses described above. In order to account for differences across stimuli (temperature, order effects and individual differences), a matrix was constructed of all responses for each voxel. The average time-course response to a given stimulus was then calculated, for each voxel, across the three blocks of stimulation and rest. Each time point in the result was an average of three values (one from each block, taken at the same time relative to the start of the stimulus in that block), and the averaged response spanned 13 time points (5 stimulus+8 rest). With five different stimuli applied to each of 15 different volunteers, there was a total of 75

recorded responses. The data for each voxel in the normalized space was therefore represented as a 13×75 matrix. A correlation between the matrices for two voxels therefore implies functional connectivity [30], across all temperatures of stimulation, order of the experiments and volunteers being studied. Voxel-to-voxel correlations of $R \geq 0.3$ were identified to show the pattern of apparent connectivity between regions. All voxels were compared in this manner, there was no distinction made between white matter or gray matter regions and no restrictions were imposed on the responses to a stimulus.

Quantitative responses, as a function of stimulus temperature and acquisition order, across all subjects, were also assessed from the spatially normalized maps of the percent signal change, β_1/β_0, from the GLM analysis. The voxels for each region were selected only from those identified as having a significant response in the random-effects group analysis, for each stimulus.

3. Results

Activity in response to thermal sensory stimulation of the right hand was detected in localized regions of the brainstem and spinal cord in all 15 volunteers. Group results show that stimulation at 29°C elicited a notably different pattern of activity than temperatures of 25°C or lower (Fig. 2).

Table 1
The fMRI signal intensity responses as a function of either stimulus temperature, or order of experiments, are indicated (in the dark grey squares) for selected regions of the brainstem and cervical spinal cord

REGIONS		localized region of C6 ipsilateral dGM	larger extent of C6 ipsilateral dGM	C6 ipsilateral vGM	C6 contralateral vGM
	RESPONSES	Temp↓ ΔSignal↑	Temp↑ Signal↑	Early Studies ΔSignal↑	Temp↓ Signal↑
C6 Ipsilateral dGM	responses are described in columns 3 and 4	(same region)		possible positive correlation	positive correlation
PAG	Temp↑ ΔSignal↑	opposite trend in PLS	same trend in PLS		positive correlation
RVM	Temp↓ ΔSignal↑	same trend in PLS	opposite trend in PLS		
LC	Early Studies ΔSignal↑	negative correlation			
Reticular Formation	Temp↑ ΔSignal↑				

The intersection of the rows and columns indicate the relationships between the responses in two regions, and whether this relationship was detected with the PLS analysis or with the connectivity analysis (based on correlation of responses). Blank squares indicate that no relationship was detected.

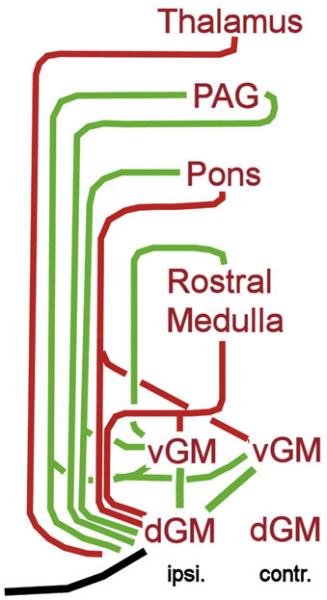

Fig. 5. Summary of results of functional connectivity calculations. Green lines indicate positive correlations ($|R|>0.3$) across all responses in all subjects (total of 75 experiments), whereas red lines indicate negative correlations. Functional connectivity is inferred from these correlations. The black line represents the location of sensory input from the periphery to the right hand. All voxels were compared within each region in the brainstem and spinal cord; no prior assumptions were imposed.

Compared to lower temperatures, this stimulus produced consistently larger areas of activity in the right cervical cord, and notable activity in the medulla, possibly in the cuneate and gracile nuclei. Stimulation of the hand at 15°C (Fig. 2) produced consistent activity (absolute T value >2.5, random-effects group analysis) in ipsilateral (right) dorsal and ventral gray matter regions around the C6 segment. Activity was also observed in contralateral ventral regions. However, these areas of activity within the C6 segment did not occur at the same R/C positions but were distributed within the segment, consistent with previous studies [31]. Activity was also observed in the regions where the RVM and PAG are located, and in the pons, in the vicinity of the pontine reticular formation (PRF). The only notable areas of negative signal changes were in brainstem regions, specifically in the pons and medulla.

Group analyses of the activity detected with each stimulus temperature demonstrated consistent responses, independent of the order of the experiments (Fig. 2), and analyses of each point in the order of experiments also demonstrated consistent responses, regardless of the temperature of stimulation (Fig. 3). However, an analysis of all data combined did not reveal any significant areas of activity that were common across all stimuli and all points in the order (not shown).

Regions of activity were contrasted with PLS analysis between lower temperatures (15°C and 18°C) and higher temperatures (22°C and 25°C) and between earlier

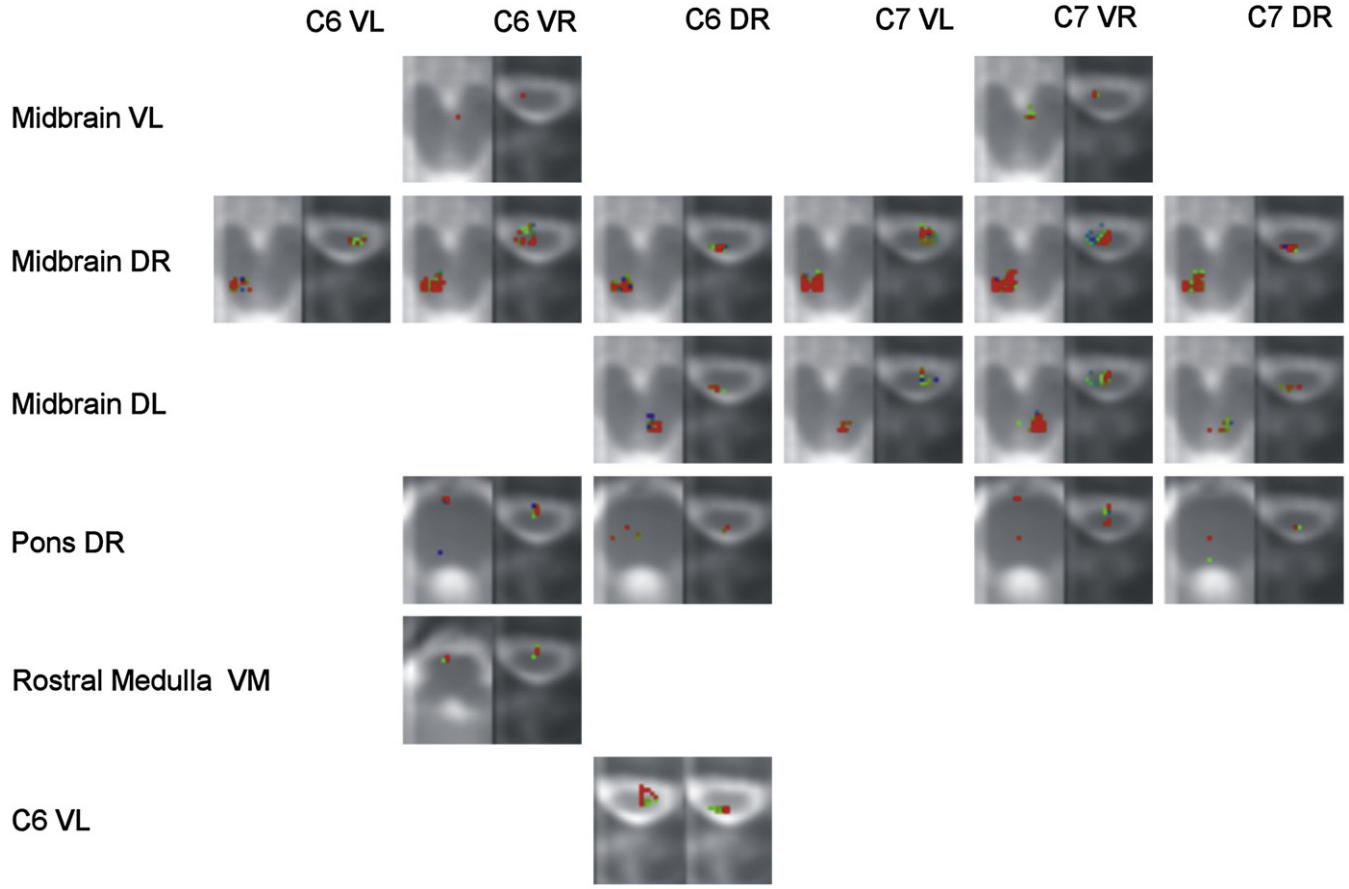

Fig. 6. Details of selected regions with positive correlations indicating functional connectivity. Each frame shows transverse slices through two regions, and the colored voxels indicate those that are significantly correlated ($R \geq 0.3$) across all 75 measured responses in 15 healthy volunteers. The colors in this figure are used to indicate the pairs of voxels which are correlated and do not indicate significance. Some colors are overwritten by others as one voxel may be correlated with multiple others.

experiments (first and second) and later experiments (third through fifth), as shown in Fig. 4. The salient features of the responses are summarized in Table 1. Activity in the cervical spinal cord was centered at the sixth cervical segment (C6), as expected. In most of this segment, the magnitude of the signal change responses were lower with lower temperatures in ipsilateral dorsal gray matter (dGM) and contralateral intermediate or ventral gray matter (vGM). At these same rostral-caudal (R/C) levels within C6, signal changes were lower with earlier experiments in bilateral dGM and vGM. However, a more localized area low (more caudal) in the C6 segment showed the opposite, with increased signal changes in ipsilateral dGM and vGM with lower temperatures and with the earlier studies.

The areas having correlated signal intensity changes (i.e., functional connectivity) across all subjects and for all stimuli are summarized in Fig. 5, and the cross-sectional distributions of selected regions are shown in Figs. 6 and 7 for positive and negative correlations, respectively. Connectivity analyses (Fig. 5) show correlations between right dorsal areas in C6 and the PAG in the brainstem and between left intermediate or ventral regions in C6 and the RVM. These areas are of particular interest with respect to the neuroanatomy as summarized in Fig. 1. In the PAG, signal changes were observed to be lower with lower temperatures and higher with earlier studies. In the RVM, signal changes showed the opposite dependence; they were higher with lower temperatures, and lower with earlier studies.

Assessments of the quantitative signal intensity responses, expressed as β_1/β_0 from the GLM, were determined for each stimulus in each subject. The values are plotted for selected regions as a function of temperature in Fig. 8. The average values across all temperatures, as a function of acquisition order, did not show significant deviation compared to the standard deviation across subjects and stimulus temperatures and are not shown. Signal intensities in the ipsilateral dGM at C6 demonstrated a generally increasing trend with higher temperatures (less intense stimuli), whereas the signal changes in bilateral vGM at C6 demonstrated a generally decreasing trend with higher temperatures. In the PAG, the signal changes were higher with stimulation at 15°C but increase monotonically from a low at 18°C to a high with stimulation at 29°C. In contrast, the signal intensity changes in the midline rostral medulla

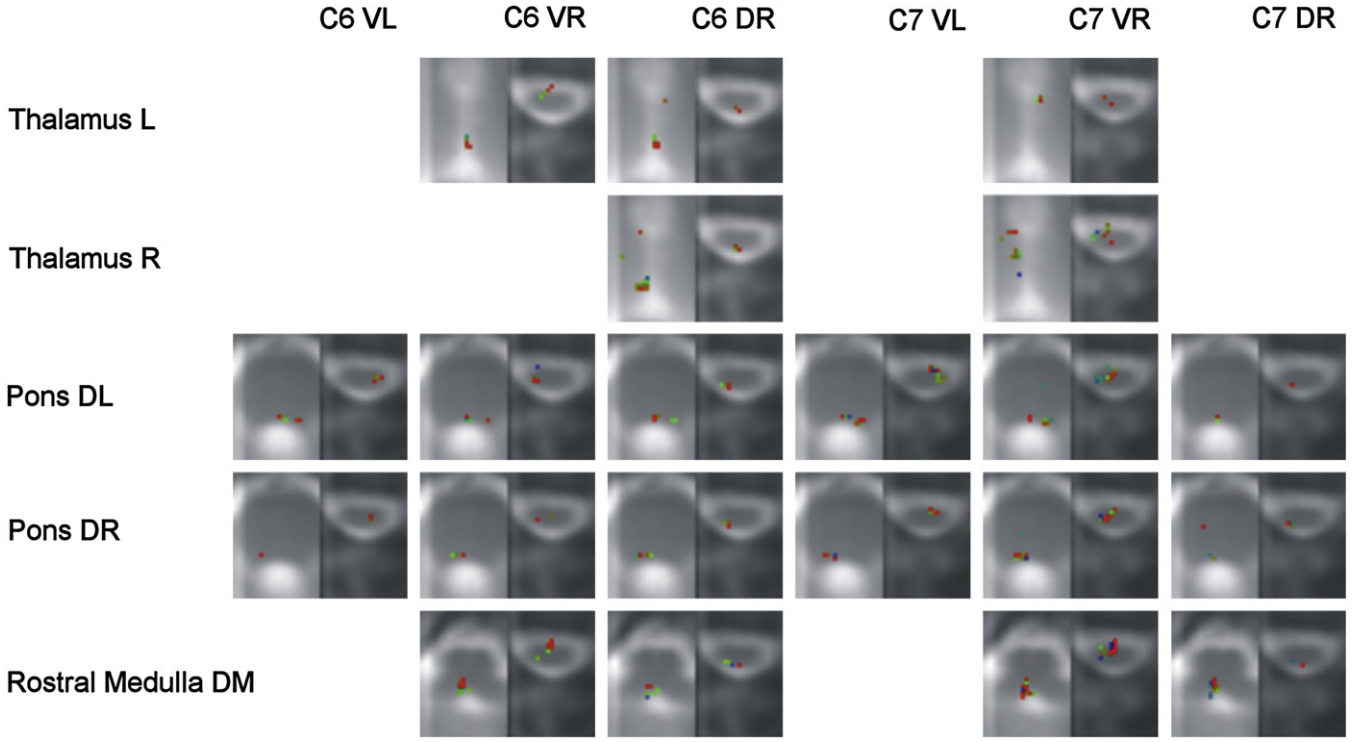

Fig. 7. Details of selected regions with negative correlations indicating functional connectivity, shown in the same manner as in Fig. 6. Each frame shows transverse slices through two regions, and the colored voxels indicate those that are significantly negatively correlated ($R \leq -0.3$) across all 75 measured responses in 15 healthy volunteers.

decreased monotonically from the response to stimulation at 15°C and 18°C (roughly equal responses), to the stimulation at 25°C, with a response that was again high with stimulation at 29°C.

4. Discussion

Interpretation of spinal fMRI results for the assessment of cord trauma or disease and effects of intervention and

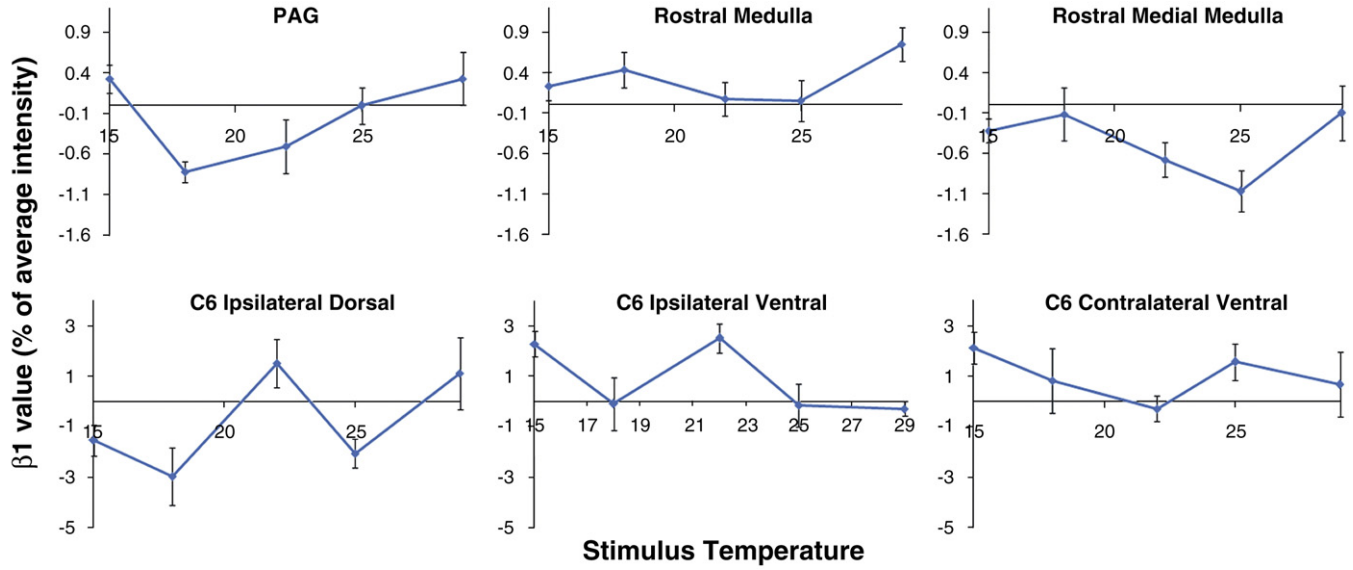

Fig. 8. Average beta values obtained from the GLM analysis, for selected regions, as a function of stimulus temperature. The error bars indicate the standard deviation of the values across the 15 volunteers, irrespective of the order of the experiments. The dependence on temperature is observed to be greater than the dependence on the order of experiments. The average responses at all temperatures as a function of the acquisition order did not show significant variation (not shown).

treatment can yield critical information about both ascending and descending pathways with unprecedented detail [3,5–7]. This builds upon studies to date that have required the use of animal models with potentially confounding effects of invasive procedures, as well as anesthesia or decerebration, or by studying the effects of injury in vivo or postmortem. Now, we can observe spinal cord function over a large extent of the human spinal cord entirely noninvasively in people who can simultaneously report their sensations. This has the potential to be a significant breakthrough for human spinal cord research. The interpretation of spinal fMRI results is still under investigation because features of the function have been detected in a number of studies that are not often described in detail of the known neuroanatomy [2,3,5,6,13,24,31–33]. These include the influences of effects such as alertness, attention and anxiety on the activity detected in the spinal cord. The process of confirming the accuracy and sensitivity of the results is ongoing, and results must be assessed in relation to known neuroanatomy.

In the present study, the group analyses show the consistent spatial distribution of the responses, and the beta values show the signal change trends as a function of stimulation temperature. The PLS analysis demonstrates the differences in responses as a function of temperature or order in the experiments, whereas the connectivity analysis shows how the responses in each region relate regardless of the stimulus or order. Hence, each of the analyses reveals different features about the neuronal response to innocuous thermal stimulation.

Activity detected in the cervical spinal cord and brainstem demonstrate spatial patterns of activity that depended on the temperature of stimulation and on the order of the experiments, as shown in Figs. 2 and 3. That the responses depend on both the temperature and the order of acquisition is also supported by the fact that there were no areas of activity common to all stimulation conditions. The response observed with stimulation at 29°C is somewhat distinct from those at lower temperatures and appears to demonstrate sensory facilitation with input from cuneate and gracile regions, as has been observed with light touch stimuli (Fig. 2) [7]. This is evidenced by the relatively large and consistent activity in the ipsilateral spinal cord at C6 and in the medulla at the approximate locations of the cuneate and gracile nuclei, despite the fact that the response was to a temperature change of only 3°C. The combined group results (Fig. 2) show that stimulation at temperatures from 25°C to 15°C elicited temperature-dependent responses in spinal cord dorsal and ventral areas. Consistent with previous studies of thermal stimuli, the areas of activity in the cervical spinal cord progressed from being primarily dorsal activity to being primarily in intermediate/ventral areas, as the temperature of stimulation was decreased. Contralateral intermediate/ventral activity was also noted and depended on the stimulus.

Analyses by means of PLS (Fig. 4) demonstrate that there were significant differences between activity elicited by different thermal stimuli, as well as differences depending on the order of experiments. The results of the PLS method provide unbiased characterization of the dependence of activity on the stimulus and order effects, across the entire group of participants, on a voxel-by-voxel basis [28]. A key difference between the PLS analysis and the random-effects group analysis [27] is that the PLS output demonstrates voxel-wise differences in signal intensity responses to different stimuli or conditions, irrespective of the signal-intensity time course model that is assumed for the response. The PLS results indicate that there were two particular areas that showed significantly higher signal changes with lower temperatures, and these were localized in the ipsilateral C6 spinal cord segment and in the rostral medulla. There were also significantly lower signal changes with lower temperatures indicated in the vicinity of the PAG, pons, rostral ventral medulla and areas of the ipsilateral C6 spinal cord segment as well. The contrast between earlier and later experiments showed a fairly general decrease in signal in much of the cord and brainstem with later experiments. However, there were specific localized areas which did not show this trend, and showed increased signal changes with later experiments in the PAG, pons, rostral dorsal medulla and at localized R/C levels of ipsilateral C5 and C6. Thus, the PLS analysis across the entire spinal cord and brainstem reveals specific responses of interest in areas which correspond well with the known neuroanatomy.

Analyses of functional connectivity, by means of correlations between all responses across subjects and stimuli indicate correspondences between specific areas in agreement with the known neuroanatomy. Positive correlations were demonstrated between the C6 and C7 ipsilateral dGM and the ipsilateral area of the midbrain where the PAG is located, localized areas of the pons and the C6 and C7 contralateral vGM. Ventral areas of C6 and C7 were observed to be correlated as well with areas in the vicinity of the PAG, the medial pons and the RVM. Negative correlations were detected between the ipsilateral dGM at C6 and C7, with the medial thalamus, pons (around the locus coeruleus) and the rostral dorsal medulla (possibly corresponding to the paragigantocellularis nucleus, PGC). Ventral areas of C6 and C7 showed negative correlations as well with the rostral dorsal medulla. An important limitation of the connectivity analysis in this study is that regions in close proximity (in the same or adjacent cord segments for example), and lying within the same sagittal slice, may have correlated motion contributions and related signal intensity changes. However, spatially separated regions such as between the C5–C8 spinal cord segments and brainstem regions will have different motion contributions and timing of cord/brainstem motion [26], and any regions spanning different sagittal image plans will have uncorrelated cord motion, enabling reliable detection of functional connectivity.

Quantitative assessments of signal changes (values of β_1/β_0 from the GLM) indicate an overall trend of greater signal changes with higher stimulus temperatures in the ipsilateral

dGM at C6, in the voxels having significant activity with each stimulus (Fig. 8). The trend appears to be opposite in the vGM regions on both sides of the cord. In the PAG and RVM, the dependence on temperature appears to be generally higher signal changes at the limits of 15°C and 29°C used in this study and lower signal changes at temperatures of 18°C, 22°C and 25°C. With increasing temperatures, however, there are trends of signal changes increasing in the PAG and decreasing in the RVM, across this 18–25°C range. The β_1/β_0 values demonstrate a greater dependence on stimulus temperature than on acquisition order, as demonstrated by the fact that averaged values as a function of acquisition order did not demonstrate significant variation relative to the standard deviations across stimulus temperatures and subjects.

This combination of analyses demonstrates that in the ipsilateral dGM in the spinal cord (primarily around C6) the response to innocuous thermal stimulation of the hand is not the same at all R/C levels within the segment. The area which demonstrated increasing signal changes with lower temperatures was observed with PLS analysis to have the same signal trends as the RVM and opposite to the trend seen around the PAG. This is assuming that the signal changes reflect primarily the neuronal input to an area, as has been indicated for the Blood Oxygenation-Level Dependent (BOLD) effect [34] and as supported by previous studies [25]. This localized area within the C6 segment was not observed to have responses correlated (positively or negatively) with those in the ipsilateral PAG, in contrast with the positive correlation observed between areas around the PAG and the remainder of the ipsilateral dGM activity at C6. However, a localized R/C extent of the ipsilateral dGM was observed to have a negative correlation with an area dorsal to the pons in the region of the locus coeruleus (LC). In the group analysis results (Fig. 2), the consistent response in the superficial dGM at C6 is seen only with temperatures of 15°C and 29°C, indicating greater input to this area specifically with these two temperatures. Descending analgesia systems include the PAG, NRM, nucleus PGC (the NRM and PGC are within the RVM), and the LC. The descending control in one pathway involves excitatory input from the PAG to the NRM, which produces inhibitory input to the dGM in the spinal cord to modulate the neural responses to painful stimuli [17]. Another pathway involves excitatory input from the PAG to the LC, which also produces inhibitory input to the dGM. The results of the present study are consistent with the input to both the RVM and dGM being greater with stimulation at colder temperatures, particularly with colder stimuli approaching a noxious level. The locus coeruleus is involved with pathways related to arousal, anxiety and stress. The results from the PLS analysis demonstrate that the region where the locus coeruleus is located had higher signal changes with earlier studies. This is consistent with this response being a component of the emotional response to being in the MRI system and, hence, varying systematically across repeated studies in the same session.

However, the larger extent of the ipsilateral dGM at C6 showed a different trend, with apparently greater input (larger signal changes) at warmer temperatures. This response may indicate sensory facilitation, with more excitatory input with warmer temperatures, or some other mode of regulation having more inhibitory input with warmer temperatures. However, there is no evidence in the present data to indicate which of these cases occurred.

The responses observed in the vGM regions were different between the ipsilateral and contralateral halves of the spinal cord as shown in Fig. 2. The group analysis shows an area of strong contralateral vGM activity at C6 with the 15°C stimulus. Connectivity analyses show that the signal intensity responses in the ipsilateral dGM and the bilateral vGM were correlated, and the PLS analysis shows consistent responses between these regions at the same R/C level within the C6 and C7 segments. Functional connectivity (Fig. 5) was also detected between contralateral vGM and the vicinity of the ipsilateral PAG, but this may be a consequence of the strong correlation between ipsilateral dGM and contralateral vGM. The analysis of signal intensity changes (β_1/β_0) shows higher signal changes with lower temperatures in the contralateral vGM. The correlation between signal changes in this area and in the ipsilateral dGM indicates that the average β_1/β_0 values are dominated by the signal changes in the areas that responded positively to stronger stimuli (lower temperatures). It is expected from the known neuroanatomy that the contralateral vGM may receive input via the reticulospinal tract, and areas of activity are demonstrated in the group analysis results where the medullary reticular formation (MRF) and PRF nuclei are located, contralateral to the stimulus. However, these areas of activity were not observed exclusively with the 15°C stimulus, and were quite prominent as well with the 29°C stimulus. The PLS analysis indicated only lower signal changes with lower temperatures in the areas of the MRF and PRF, which is not consistent with the contralateral vGM response. Thus, the evidence from this study indicates that the contralateral vGM activity arises from input from the ipsilateral dGM. It is expected to be modulated by input from the reticular formation, but the results of the present study do not demonstrate this input over the stimulus temperature range studied. This conclusion is consistent with previous studies of patients after spinal cord trauma who also had significant contralateral vGM activity [32], which appeared to be *greater* with complete injuries compared to incomplete injuries. This would also indicate a local source of input to the contralateral vGM, with the intact spinal cord having descending modulatory input to the ipsilateral dGM and possibly also the contralateral vGM.

The responses to the thermal stimulation in the ipsilateral vGM appear from the group analysis to have little dependence on the stimulus temperature. However, the quantitative analysis indicates that the β_1/β_0 values had a trend toward increasing values at lower temperatures. The

PLS analysis indicates higher signal changes with earlier studies and does not demonstrate a dependence on stimulus temperature in this region over most of the C6 and C7 segments of the spinal cord. Connectivity analyses indicate correlations with areas in the vicinity of the PAG and in the pons and rostral medulla. These areas in the pons and rostral medulla may correspond with reticular formation nuclei. Combined, these results indicate a variable response in the ipsilateral vGM that depended on the acquisition order and likely depended as well on the stimulus temperature. The dependence on acquisition order reflects descending modulation of the response and, therefore, may also have been influenced to a greater degree than the other areas of activity by intersubject differences in cognitive and/or emotional influences of being inside the MRI system. This is also consistent with the possibility of input from the reticular formation [9].

5. Conclusions

The contributions to the activity detected in the cervical spinal cord that can be concluded from the present study are summarized in Fig. 9. Activity in the ipsilateral dGM, in response to innocuous thermal stimuli, reflects the net result of sensory input to the cord and modulation from the descending analgesia system (via the RVM) and from affective (emotional) components of sensation (via the LC). Similarly, activity in the ipsilateral vGM depends on that in the dGM plus descending modulation from affective components (via the PRF). In contrast, contralateral vGM activity reflects local input from the ipsilateral dGM. Descending modulation of activity in the contralateral vGM via the reticular formation cannot be ruled out, but was not observed with 15–29°C stimuli used in this study. The initial hypothesis is therefore demonstrated to be true on the side of the cord ipsilateral to the stimulation, but not on the contralateral side.

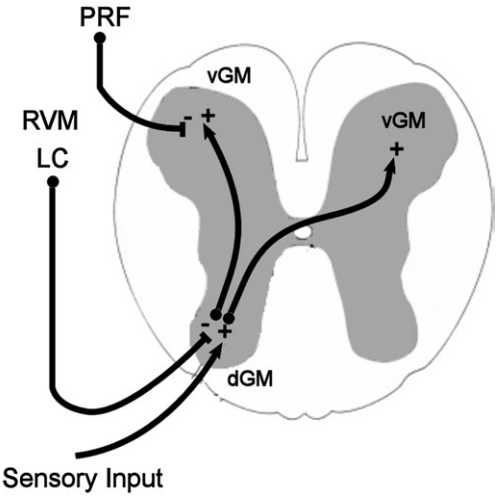

Fig. 9. Summary of interpretation of activity observed in the sixth cervical (C6) spinal cord segment. Inputs to the spinal cord gray matter, with its characteristic butterfly shape, are indicated for sensory input to the right dorsal horn of the gray matter, with the schematic of the anatomy shown in radiological orientation. Activity in response to innocuous thermal stimuli in the ipsilateral dGM reflects the net result of sensory input to the cord, and modulation from the descending analgesia system (RVM) and from affective (emotional) components of sensation (LC). Similarly, activity in ipsilateral vGM depends on that in the dGM plus descending modulation from affective components via the PRF. In contrast, contralateral vGM activity reflects local input from the ipsilateral dGM. Descending modulation from the reticular formation cannot be ruled out but was not observed with 15–29°C stimuli applied in the present study.

The order of the experiments is linked to systematic decreases over time in the volunteers' levels of stress and anxiety, and increasing boredom. Group fMRI results demonstrated significant dependences on the order of the experiments, most notably in the LC. These results also demonstrated significant dependences on the stimulus temperatures in the observed regions of activity in the brainstem and cervical spinal cord, showing both inhibition of pain and motor responses with lower temperatures, and apparent sensory facilitation with stimulation at the warmest temperature, 29°C.

This study reveals, for the first time, the complete network of neuronal function in the cervical spinal cord resulting from innocuous thermal sensory input and descending modulation in human volunteers who can simultaneously report their sensations. The reliability and sensitivity of the results obtained are demonstrated by the observation of specific consistent responses to changes in temperature and time spent in the MRI system (i.e., emotional factors), across healthy participants, and by the detection of functional connectivity between anatomical regions that are known to be relevant to pain and thermal sensory function. The results of this study will enable accurate interpretation of spinal fMRI results, including the potential to identify altered ascending and descending pathways as a result of disease or trauma to the cord. They also demonstrate the potential of spinal fMRI to provide a view of spinal cord function in humans without the complications of invasive procedures or anesthetics. This view reveals anatomical and functional details of distributed networks in the human brainstem and spinal cord that produce a net sensory response, in unprecedented detail, and has implications for the future study of conditions such as neuropathic pain and regeneration after spinal cord injury.

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Appendix A. PLS Analysis

Group analyses were carried out with spatially normalized data [24] by means of the PLS method as described by McIntosh et al. [28,35]. The data from each experiment were averaged over the 3 stimulation periods to generate a single mean response of length t, equal to 117 s (the duration of one 45-s stimulation period followed by a 72-s baseline period). The responses to the higher (22–25°C) temperatures were compared to those of the lower (15–18°C) temperatures and earlier (first and second in the order) were compared with the later experiments, by computing the average difference between the responses. A spatially normalized region-of-interest mask was used to select the m voxels which represented the brainstem and spinal cord. The average differential responses were represented in a matrix, **Y**, of 2 rows and $m*t$ columns, and the principal components of this matrix were determined. The principal components are the voxel "saliences" as described by McIntosh et al. [28] and represent the weight of the covariance contribution of each voxel. Positive weights imply increasing signal intensity changes with increasing subject expression of a covariance pattern, while negative weights imply simultaneous decreasing signal intensity changes with increasing subject expression. The significance of the voxel saliences were determined with a bootstrap method, in which the original data were resampled by substitution, without changing the order of the experiments for each volunteer. For example, the data for Volunteer 2 would be replaced with that from Volunteer 1 in one permutation. A total of 211 such permutations were analyzed and the standard error of the saliences were determined. The ratio of the salience to its standard error is termed the "bootstrap ratio" and provides an estimate of its significance. This analysis constitutes a single statistical test and so corrections for multiple comparisons are not required [28].

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